

An accelerator worth fighting for

The Large Hadron Collider project faces new financial obstacles. Some proposed solutions to its problems are implausible. Others can surely be found, but will need great skill from CERN's management in limiting the damage to the laboratory.

WITH the best will in the world, people can commit themselves to an agreement only to find that circumstances change and that what looked like an unbeatably reasonable deal has turned into a millstone. Resolving the dilemma may amount to a battle between expedience and honour but, whatever one then does, somebody will be upset. That is where the member states of the European Laboratory for Particle Physics (CERN) will soon find themselves, following new pressure from some of them to curtail its costs even more than it has done already.

But what circumstances have changed since 1994, when the proposal to build CERN's next major accelerator, the Large Hadron Collider (LHC), was unanimously approved by its member-state governments? Certainly, the case for doing this \$2-billion piece of physics has not weakened. The means by which matter acquires mass remains a profound unknown. The best theoretical answer we have applies to obscure particles that carry one of the fundamental forces — the electroweak force. Their masses are thought to arise by the action of a hypothetical particle, the Higgs boson, of which there may be several varieties. Although there is plenty of other fundamental physics that the LHC is expected to tackle, its most important foreseeable role will be to explore 'the Higgs sector' of the standard model of high-energy physics.

In the absence of the United States' late Superconducting Super Collider project, the LHC will provide unique access to Higgs processes. CERN's 19 member countries in western and central Europe, and subsequently Russia, India, Canada, Japan and, almost certainly, the United States are willing to find the money over the next ten years or so to carry out such science. Rightly, that fundamental commitment remains strong.

What also has not changed is CERN's persistent and conspicuous success at achieving what its member states demand of it: currently, pursuing a diminishing budget by losing staff, closing facilities prematurely and trimming overheads. What is more, as part of its consideration of joining the LHC programme, the US Department of Energy issued in June a glowing assessment of CERN's performance, finding the LHC project to be functioning well, under a strong management team, and on track for possible completion in 2005.

Despite that, the formula by which member states' contributions are calculated has produced a drastic increase in the annual cost of membership to the United Kingdom, as a result of changes in relative economic performance and currency exchange rates. The United Kingdom is not alone in experiencing pressures on its domestic particle-physics programmes, although it is only now that other countries are beginning to make noises about the situation. Nevertheless, CERN would be justified in playing the injured innocent: ahead of the 1994 approval of the LHC, it presented all the costs up front — both of the accelerator and of its detectors, which are being funded predominantly from domestic programme budgets — and conditions of membership have not changed.

A critical new factor is the determination of Germany's government to adopt budgets aimed at meeting criteria established by the 1992 Maastricht Treaty, necessary to the establishment of a single European currency. The severe consequences for European science of that policy were revealed in our pages last week (*Nature* 382, 285; 1996). Perhaps it is a political inevitability that domestic

science programmes should fare better than international collaborations, which attract few votes. That would be consistent with the success of Germany's national high-energy facility DESY in being awarded virtually level funding next year, in contrast to the 9.3 per cent cut likely for CERN.

Despite CERN's relatively blameless track record, the political pressures confronting its council could well prove irresistible. So what are the obstacles to possible ways forward, and are they as insurmountable as some claim? One option is to try to turn CERN into a truly international facility — to scrap its European identity, in other words. To hard-pressed member states, spreading the costs may sound like good sense.

One obstacle to that course is probably spurious. Despite references to its European role, the constitution of CERN does not explicitly prohibit non-European countries from becoming members. It would require a unanimous vote of member states to permit it, and Europhilia (or Anglo-Saxophobia) may be too strong in some for the possibility to be contemplated. But the genuine obstacle is that it is not in the interests of countries such as the United States or Japan to join CERN as members. Their high-energy physics communities are dominated by researchers whose interests lie in domestic facilities, such as KEK and SLAC. To join CERN commits members to paying establishment costs, including pensions. And even if their own finances were ideal, the US Congress and the Japanese Diet seem unlikely to favour a scheme that has never been canvassed before, and which would be a blatant attempt to get the Europeans out of a financial hole.

Given the political realities, another alternative may prove to be the only one: a revision of the LHC programme. Given CERN's overheads, slowing down construction of the accelerator can only increase its total cost, but may reduce annual costs to members. Changes in the deployment of the massive and expensive detectors may be possible, though at the risk of reducing the scientific value for money for the participants. If the priority for LHC physics remains sufficiently high, CERN may also be forced to think hitherto unthinkable thoughts about its other programmes, although managers will say that everything possible, draconian or otherwise, has already been done.

In considering these problems over the next few weeks and months, the governments of CERN's members states should keep in mind that maintaining the confidence of non-member states in Europe's capacity to deliver is vital. Meanwhile, European high-energy physicists — or at least those participating in the LHC — and their institutions will need to keep all their lobbying wits about them.

But the real pressure will be on CERN's own management and scientists. They have a track-record, an intellectual authority and a public profile that stand them in good stead. Despite unfavourable winds from member states, they have no reason to give an inch — yet. That is especially true given that there is no easy inch to give. But there will come a point later this year when member-state representatives and CERN's leaders will once again be at loggerheads over the finances. If the governments insist on 10 per cent cuts, the LHC project may collapse. If compromise is possible, the LHC's future will surely depend on the political astuteness of CERN's management and influential supporters. □



Britain draws up 'superleague' plan for leading research universities

London. Britain's Conservative government is planning to propose that a significant portion of its research budget is concentrated on a 'superleague' of nine or ten élite universities, almost 10 years after rejecting similar proposals as too politically sensitive.

Gillian Shephard, secretary of state for education and employment, will suggest the move to Sir Ron Dearing, a government education adviser who is conducting a review of higher education, according to government officials quoted in the British press.

The proposals will affect funding only from the three higher education funding councils, and not the research councils. Precise eligibility criteria for the superleague have not been revealed. But reports suggest that it will contain a fluctuating membership, based on research results rather than research income. Universities outside the league will be promoted to it if they reach the appropriate standard; those that do not perform adequately will be relegated.

Ten years ago, David Phillips, chairman of the now defunct Advisory Board for the Research Councils (ABRC), made similar suggestions in a report for the government of prime minister Margaret Thatcher (see *Nature* 328, 280; 1987 & 330, 3; 1987). The ABRC report forecast continued dwindling research income, and proposed dividing up universities into three types: research-only, research and teaching, and teaching only — the so-called 'RTX' formula.

The report was heavily criticized by the

scientific community. It was later rejected by Kenneth Baker, then education secretary. But Phillips, now a member of the House of Lords Select Committee on Science and Technology, says the report's adoption was inevitable, as the twin goals of funding both aspiring and élite research departments "required a lot of money, which was never going to come".

A spokeswoman for the education department points out that Shephard's reported statements are a continuation of the government's policy of concentrating research funds among a select number of departments pursuing 'world class' research. Already more than half of research funding from the Higher Education Funding Council for England goes to just 14 out of more than 100 university institutions.

The government's proposals have received a mixed reception, even among candidates for the superleague. Both supporters and critics acknowledge that they are designed to preserve the main citadels of research excellence at a time of sharp reductions in higher education funds, particularly for research equipment.

Alan Swanson, pro-rector for educational quality at Imperial College in London, says the suggested policy amounts to a belated recognition of the status quo. But David Livesey, secretary general of the faculties at the University of Cambridge — who supports selectivity at the departmental level — warns that a superleague of universities

could erode Britain's research base.

Swanson points out that an effective superleague has been in operation for "donkey's years". Ideally, the government should provide more money for research, he says. But, "given that this is not going to happen, it is better to concentrate the limited funds on a few institutions."

But Derek Burke, former vice chancellor of the University of East Anglia — a leading research centre for environmental sciences and meteorology, but unlikely to become a member of the superleague — describes such a view as "arrogance" and "power play by the big guys". Burke points out that turning



Shephard: continuing a policy of concentration.

ing off the funding tap will wipe out important pockets of high quality research in lower-tier universities.

Concentrating funds in a superleague could also harm the government's Technology Foresight exercise, he warns, through which the government funds research to enhance wealth creation and the quality of life. The success of the exercise, he says, relies on research excellence at smaller, newer universities.

The government's proposals are also opposed by the Association of University Teachers (AUT), the labour union which represents university staff. Paul Cottrell, assistant general secretary of the AUT, says that they effectively undermine the government's earlier decision to grant university status to all the former polytechnics.

The proposals mirror the conclusions of several independent reviews of postgraduate education funding. These include a review chaired by Martin Harris, vice chancellor of the University of Manchester (see *Nature* 381, 266; 1996), and a report from a working party chaired by David Harrison, Master of Selwyn College, Cambridge.

But Peter Collins, head of science advice at the Royal Society, points out that these reviews advocated concentrating funds at selected departments, rather than institutions. The study that inspired the ABRC report — a review of Earth science departments in 1987 chaired by Sir Ronald Oxburgh, former rector of Imperial College — also recommended the tiering of individual departments, rather than institutions.

Ehsan Masood

BSE transmission data cause confusion

London. The possibility that bovine spongiform encephalopathy (BSE) might be transmitted from cattle to sheep caused widespread concern and confusion last week. Franz Fischler, the European Union's Agricultural Commissioner, responded to what he described as "experimental evidence" by calling for a ban on various sheep and other ruminant tissues entering the food chain.

Research by the UK Institute of Animal Health has found that sheep selectively bred for resistance to natural scrapie, a disease in the same family as BSE, could still be infected by brain extracts from cattle suffering from BSE. Tissues recovered from one such sheep, out of six fed the infected material, induced a disease in mice with incubation periods and pathology closely resembling those caused by direct transmission of BSE from cattle to mice.

Although the pathways of the disease seem to be different in cattle and sheep — only sheep showed infectivity in the spleen — the results have been taken to indicate a theoretical risk. Douglas Hogg, Britain's agriculture minister, says he is treating this with an "abundance of caution", recommending that sheep heads are destroyed in the same way as those of cattle.

Chris Bostock, head of molecular biology at the institute, describes the ban as a "sensible precaution". But he points out that, due to the similarities between BSE and scrapie, distinguishing between them when diagnosing sheep may be very difficult.

But concerns that scrapie in sheep could be masking BSE have come from "people speculating", says Francis Anthony, a spokesman for the British Veterinary Association. Ginny Page

Australian investors lose tax incentives on innovation

Sydney. In its first significant change to Australian research policy, the new Coalition government has cancelled a scheme that has used tax incentives to encourage investors to form 'syndicates' for commercializing the results of research.

Australian researchers and government departments have long been trying to boost local industry and to increase the low level of investment by financial institutions in science and technology. In 1986, the former Labor government introduced an attractive 150 per cent tax rebate on approved expenditure by industry on in-house research and development. This incentive, worth A\$550 million (US\$433 million) in 1994-95, will be retained and "fine-tuned".

The syndication scheme — believed to be unique to Australia — was introduced in 1987. It allowed tax concessions of 100 per cent on losses to investors on money that was gathered by financial houses into lump sums for use by companies in the commercialization of discoveries. On the recommendation of the Bureau of Industry Economics, the scheme has now been cancelled by the Treasurer, Peter Costello, and the industry minister, John Moore.

Any debate or lobbying to retain the scheme was forestalled by announcing the decision on the day the bureau's report was released. Citing four unnamed examples of alleged abuses, the ministers claimed that syndication had "become focused on tax minimization rather than the provision of genuine research and development".

They added that the government was concerned about the amount of tax revenue lost through tax concessions, which

amounted to A\$255 million for 217 syndicates in 1994-95.

The international quality of biomedical research in Australia's universities and public agencies attracted many of the syndicates. The University of Sydney, for example, collaborated with the Commonwealth Scientific and Industrial Research Organisation, the AMRAD company and Macquarie Bank (which raised A\$20 million over three years) to develop an antiviral drug to treat a strain of hepatitis.

Graham Johnston, a pharmacologist involved in the collaboration, says that it has been one of the most exciting times of his research career. It has brought together three groups of partners — researchers, industry people and financiers — for a targeted programme. Johnston and others had been calling for the syndication scheme to be retained, but with tighter rules. The Federation of Australian Science and Technology Societies wanted a peer-reviewed scheme of direct funding to be added.

The government says it will now "consider" a new programme of grants or loans. Urgent consultations have begun to devise a scheme before the release on 20 August of its first budget, which is predicted to bring cuts of A\$4 billion.

Although existing syndicates will be able to continue, the withdrawal of tax benefits has already prompted several potential investors to cancel negotiations over investments in local research efforts, and has led some observers to predict that there will be an increase in the number of Australian innovations that are now developed by overseas companies. **Peter Pockley**

US industry lobbies for extension of \$3bn tax credit schemes

Washington. The extension of two research tax credits worth about \$3 billion are at stake in a bill being finalized this week in the US House of Representatives and Senate. President Bill Clinton has urged Congress to send him the bill to sign into law before it goes into summer recess.

The two tax credits — one to entice companies to expand their general research and development, the other to encourage the development of drugs against rare diseases — lapsed in 1995 and 1994 respectively. A minimum wage bill passed by the Senate in June extends the two credits for 18 months, beginning on 1 July 1996. But the version of the bill passed by the House, which raises the minimum hourly wage from \$4.25 to \$5.15, contains no such provisions.

Industry has been lobbying not only for the inclusion of the measures in the final bill, but also for amendments to enable companies to claim the credits retroactively.

The 'orphan' drug credit allows companies to subtract from their taxes 50 per cent of the costs of human clinical testing of orphan drugs, defined as those with disease populations of fewer than 200,000. They are so-called because advocates say the drugs were "orphaned" in the past by pharmaceutical companies.

The research and experimentation credit allows companies to subtract from their taxes 20 per cent of expenditure on new research and development.

Influential members of Congress have joined groups such as the Biotechnology Industry Organization (BIO) in pushing for the retroactive extension of the credits, a position which the Clinton administration also supports. In June, a bipartisan group of 33 senators wrote to leaders of the negotiations, calling the lapse in the credits "a troubling signal" to industry and a threat to US competitiveness that would "severely impact companies' commitment to research".

But Senator William Roth (Republican, Delaware), chairman of the Senate Finance Committee and the lead negotiator, opposes retroactive extension. "It is to be used as an incentive and to make it retroactive would defeat the purpose," says Ginny Koops, a spokeswoman.

And fiscal conservatives are concerned about the costs of extending the credits. Congress's Joint Committee on Taxation puts the cost of the research credit extension at \$2.9 billion, and the orphan-drug credit extension at \$44 million.

Orphan drug developers are hoping for the retention of a provision in the Senate bill that would allow companies to claim the credit up to 14 years after research costs were incurred. **Meredith Wadman**

Indian budget boosts AIDS research

New Delhi. The Indian government has allocated the equivalent of US\$1,516 million (5.4 billion rupees) for research and development in the 1996-97 budget, about \$60 million more than last year. But scientists say this represents a cut in real terms because the value of the rupee has fallen by 6 per cent.

Funding for AIDS research and control has been doubled to \$40 million, however, more than 40 per cent of the total allocation for medical research. Research in herbal medicine has also received a boost, with a provision of \$12 million.

Few new research projects have been announced. The Department of Biotechnology will set up a \$2.5-million centre for DNA fingerprinting and diagnostics in

Hyderabad, and the space department has announced plans to build and launch next year a \$12-million satellite, OceanSat, to monitor the resources of the seas around India.

Although scientists are disappointed by the budget, they have welcomed the government grant of \$8.6 million to the technology development fund. This was created two years ago to help commercialization of home-grown technologies. They have also welcomed the announcement of a grant to modernize the 80 or so industrial and agricultural laboratories. The amount has not been specified but it will be equal to what the laboratories earn through consultancy services and commercialization of the technologies they develop. **K. S. Jayaraman**

US agencies 'can fight' declining budgets

Washington. US science agencies will be able to win larger budgets in future years than existing projections suggest, officials of the Clinton administration told a congressional hearing investigating the impact of the projections.

Dan Goldin, the administrator of the National Aeronautics and Space Administration (NASA), told the hearing that he believed that President Bill Clinton had "empowered" him to "go into the Office of Management and Budget (OMB) and fight" for more than the projections.

These would cut NASA funding from \$13.9 billion this year to \$11.6 billion by 2000. The OMB is the powerful branch of the administration that compiles the president's annual budget proposal.

Goldin and other agency heads were brought before the Science Committee of the House of Representatives on 24 July for a long-delayed and politically charged hearing (see *Nature* 382, 7; 1996). The committee chairman, Robert Walker (Republican,

Pennsylvania), sought to prove that the administration was guilty of dishonest budgeting. Neal Lane, director of the National Science Foundation (NSF), and Martha Krebs, director of the Office of Energy Research at the Department of Energy, also testified.

Walker believes that the Republicans who control Congress have been unfairly attacked for planning to cut science budgets over the next six years, while the Clinton administration is projecting cuts almost as deep. He criticized the three officials for "going around the country attacking the Republicans' budget plan" and arguing that Clinton's own long-term plan does not matter.

The administration and Congress have published rival plans to balance the federal budget by 2002. According to an assessment by the American Association for the Advancement of Science (AAAS), both plans imply deep cuts in spending on research and development (see figure, below left).

At a hearing of the Science Committee the day before, Al Teich, an AAAS budget analyst, acknowledged that "out-year projections [those projecting more than two years ahead] have not been reliable" in previous years. But he testified that "they are worth our attention because they tell us about the problems we face and the choices we will have to make".

Comparing the two plans,

Teich said that, in general, NASA and the NSF were ahead in the congressional plan while the energy and commerce departments did better under Clinton's. Teich added that research and development "has consistently averaged about 14 per cent of all discretionary spending" and that this was likely to remain the case. Both plans would cut discretionary spending by one-fifth by 2002.

Senator Kit Bond (Republican, Missouri), who chairs the appropriations subcommittee that funds NASA and NSF, testified that one of the space agency's main areas of activity "would have to go" under the Clinton plan. That could mean axing the space station, the shuttle, the Mission to Planet Earth, or space science.

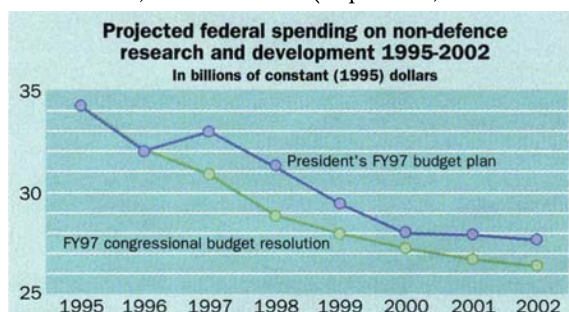
But Goldin said: "I don't believe it makes sense to cut back on programmes based on anticipated problems. OMB expects us to fight [for more money] and OMB will not be disappointed."

George Brown (California), the senior Democrat on the committee, said that "both budgets would continue a steep decline" in US research spending as a percentage of gross national product. He added: "That is abysmal and reprehensible."

But Harold Volkmer (Missouri), another senior Democrat, defended Clinton's commitment to a balanced budget. He accused Walker of conducting "a witchhunt" and of using the committee for party-political ends "to make the president look bad".

Colin Macilwain

Source: AAAS



Internet mentors seek to bring more women into science

Boston. Plans to launch a nationwide science 'mentoring' programme for women students, using electronic mail and the Internet, are to be discussed next week at a meeting of representatives of leading US universities and colleges.

The programme would be based on a successful one-year pilot demonstration at Dartmouth College in New Hampshire. Dartmouth's 'E-mentoring' project links female students with mentors, almost all of whom are women employed in science-related industries.

The intention is to devise a plan to expand the project to include 100 universities and 5,000 students over a five-year period from autumn 1997. Colleges to be represented at next week's meeting in Boston include Dartmouth, Carnegie Mellon, Cornell, Purdue, Stevens Institute of Technology, and the Universities of Washington, Michigan, and California at Berkeley.

According to Mary Pavone, director of the Women in Science Programme at Dartmouth, the approach "works particularly well for this school, given our remote

location and the fact that our entire campus is networked". She adds: "We have a student culture based largely on e-mail communications." E-mail, says Pavone, is an economical, convenient and non-intrusive method for students to correspond with their mentors, offering an easy way to bridge the gap between the different lifestyles and schedules of students and working professionals.

In the Dartmouth programme, 35 science or engineering students were assigned mentors, who offered them advice about choosing courses or finding a job, as well as providing encouragement to persist in science and engineering. Through these interactions, Pavone says, "students gain a perspective they couldn't get by talking to a faculty member, parent or friend".

Carol Muller, associate dean of engineering at Dartmouth, claims: "The reinforcement that students get from outside the university can be as important, or more important, than what they get from inside the university. This programme offers them outside encouragement and support." She

says that 60 per cent of graduates in science and engineering go on to work in industry.

The motivation to expand the programme stems from the fact that female students are "under-represented" in physical and quantitative sciences, and particularly in engineering, explains Muller, who is leading the initiative. Pavone cites recent US figures showing that women comprise 16 per cent of the people working in science and just 8 per cent in engineering. This imbalance, she says, is due to "subtle and not-so-subtle forms of discrimination, as well as early socialization".

A nationwide 'E-mentoring' programme would be administered by the Women in Engineering Program Advocates Network. The organization's president, Suzanne Brainard, director of the Women in Engineering programme at the University of Washington in Seattle, says a national programme could have an "enormous" impact.

The Alfred P. Sloan Foundation has provided a grant to support the planning process for establishing a national programme.

Steve Nadis

Medical developments get small investors' backing in Canada

Ottawa. In an unusual financial experiment designed to ensure that Canadian discoveries are exploited at home rather than abroad, medical scientists have set up a venture-capital fund. In under 18 months the fund has attracted almost C\$190 million (US\$140 million) from 47,000 individuals, each investing an average of C\$4,000.

The Canadian Medical Discoveries Fund (CMDF) arose from an initiative of the Medical Research Council (MRC) of Canada. Its partners are MDS Health Ventures Capital Corporation, Talvest Fund Management and the Professional Institute of the Public Service of Canada.

Calvin R. Stiller, a physician from London, Ontario, and chairman of the fund, says he expects investment in what is already one of the top ten venture-capital funds in North America to reach C\$500 million in three years.

The fund resulted from a study commissioned by the MRC to find out why, although the quality of science in Canada compares well with that in other developed countries, few Canadian discoveries are successfully commercialized within its borders. The study concluded that the reason was a lack of venture and pre-venture capital.

The fund takes advantage of Canadian legislation that provides small individual investors with significant tax incentives to invest in venture-capital funds, provided they do not withdraw their money within specific terms, typically five to eight years.

The CMDF was created under this legislation with the aim of funding the commercialization of pre-discovery, discovery and post-discovery medical research. The MRC acts as a strategic partner, helping to identify projects for funding and monitoring their progress.

Nine projects have so far been funded, most of which have or had some connection with the MRC. They include investments in companies researching apoptosis, novel therapeutics to inhibit cancer spread and to destroy malignant tumours, 3D ultrasound-imaging systems, anti-infective compounds aimed at drug-resistant bacteria, an automated system for analysis of pap smear tests, three compounds for osteoporosis treatment, and vaccines against infectious diseases and cancers. Individual grants range from C\$1 million to C\$13.5 million.

CMDF provided much of the stimulus for the doubling of venture capital that occurred in the year 1994-95 alone.

Two months ago the fund created University Medical Discoveries Inc., which will provide universities with money and expertise to patent discoveries quickly at no cost to themselves.

David Spurgeon

Liability clause blocks talks on biosafety protocol

London. Developing countries ended a week of negotiations in Aarhus, Denmark, last Friday, divided over the content of proposed internationally binding regulations governing the use of genetically modified organisms (GMOs).

Member countries of the G77 group last year agreed to proposals for a new international biosafety law (or protocol) regulating the import and export of GMOs, referred to as 'transboundary movement' (see *Nature* 378, 326; 1995).

Last week's meetings were intended to start negotiations on the protocol's content. But a breakaway faction, including Malaysia, Ethiopia and Mauritius, tried to widen the scope of transboundary movement to cover handling and use of GMOs.

This group is also lobbying for a clause that provides compensation where GMO release damages human health and the environment. They want a related clause that assesses — and possibly compensates for — the impact of biotechnology on traditional agriculture.

"The question of liability and socio-economic impact of GMOs is a matter of grave concern to less developed countries," says A. H. Zakri, head of Malaysia's delegation to the United Nations (UN) biodiversity convention, and deputy vice-chancellor of the Universiti Kebangsaan Malaysia, near Kuala Lumpur. "I know that this is not biosafety *per se*, but, since GMOs present a potential risk to our environments and economies, we would like this to be included in the protocol."

But the demands are being resisted by the European Union (EU), industry representatives such as the BioIndustry Association, the lobby group for British biotechnology companies, and some developing countries. They feel that stronger regulations will inhibit emerging and established biotechnology industries. They could also limit the attractiveness of developing countries to multinationals that may want to invest in bio-prospecting or GMO field trials.

Malpede Diego, head of the Argentine delegation and a member of Argentina's mission to the UN in Geneva, says: "We think it is premature to discuss liability in GMO release. We understand the concerns relating to socio-economic consequences [of the use of GMOs]. But this is a large and complex issue. It is hard to see why this should be included in a biosafety protocol."

The European Union has taken a similar line. A statement issued to coincide with the Aarhus meeting said that adverse environmental effects from the transboundary movement of GMOs were "unlikely", on account of their "contained

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Seed change: G77 countries are concerned over impact of biotechnology on agriculture.

use and separation from the environment".

The Denmark meeting brought together the *ad hoc* group of experts on biosafety convened by the 150-member UN biodiversity convention to write a draft protocol. All the delegates agreed that any exporter would need to obtain the prior informed agreement of the country to which GMOs are to be sent. The exporting organization would need to comply with local safety regulations.

But there was further disagreement on the definitions of terms to be used in a protocol, including the phrases 'living modified organism' (LMO), which is now used in all official documentation, and 'transboundary movement' of LMOs.

The EU says LMO refers to "any biological entity capable of replication or of transferring genetic material in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination". But the breakaway G77 countries say that LMO must also cover any product that arises from a process involving genetic modification. Until this issue is resolved, the experts' group will not be able to begin classifying which LMOs pose a health or environmental hazard.

The EU also suggests that the protocol could be restricted to "unintended" movement of modified organisms between states. Some aspects of authorized movement of LMOs, according to the EU statement, may already be covered by UN rules on the transport of dangerous goods.

A draft text of the protocol is expected to be completed by 1998. One hundred and fifty-two countries and the EU have signed and ratified the convention, which was opened for signature at the Earth Summit in Rio de Janeiro in 1992. The United States has not yet ratified it.

Ehsan Masood

Livermore to get \$100m supercomputer

Washington. President Bill Clinton announced last week that the US Department of Energy is to buy the world's most powerful supercomputer from IBM.

The \$100-million machine will start operating in 1998 at Lawrence Livermore National Laboratory in California. It will be used to simulate nuclear explosions in the absence of testing. But Clinton added that "it can quickly be switched over to civilian uses as well".

Energy department officials say that about 20 per cent of the machine's capacity will be used for classified-weapons work and 20 per cent for non-classified scientific use. The remaining 60 per cent will be interchangeable between the two.

Bruce Tarter, director of Lawrence Livermore, said that the machine would help US weapons laboratories to regain their world leadership in the use of supercomputers. In the 1960s and 1970s, the weapons laboratories had the fastest machines in the world. But the arrival of parallel processing and distributed computer workstations in the 1980s marginalized their lead. "We weren't special in that context," Tarter says.

Now the energy department's multi-billion-dollar Accelerated Strategic Computing Initiative (ASCI) aims to boost supercomputing power in the laboratories

of the world for leadership in science and technology, he said.

"To stay on top, I've done everything I can to increase our commitment to science and technology at every level, and especially at the universities. No investment we've ever made has paid off more, in jobs and security.

"If we really want the America of our dreams, we must have research and development that's the best in the world."

The president was speaking at the National Science and Technology Medal awards ceremony at the White House on 26 July. He awarded a special Technology Medal to the widow of Ron Brown, the commerce secretary who died on a trade mission to Bosnia earlier this year, and pledged to defend the controversial Advanced Technology Program (ATP) in Brown's honour.

Clinton said that congressional attacks on ATP were based "more on ideology than on the evidence". He claimed that the programme had "forged remarkable progress in the private sector".

Clinton is campaigning for re-election not on the basis of any broad theme but through a steady stream of announcements, each addressing the concerns of some small constituency. The supercomputer announcement, which received nationwide television coverage, was the scientists' turn.

The supercomputer procurements will total \$900 million in the first five years of ASCI alone. But they will raise international questions about the extent to which the United States is still subsidizing its supercomputer industry through the nuclear

weapons programme.

The administration says it wants an open market in supercomputers, but Japanese and European suppliers have yet to make a single sale to the US federal government (see box, below).

Jack Gibbons, Clinton's science adviser, said that the administration "believes in international competition in business, including the supercomputer business" and predicted that Japan would "sell some over here soon".

He criticized Representative David Obey (Democrat, Wisconsin) for his attempt to dock the salaries of US government officials who tried to buy a supercomputer from NEC (see *Nature* 382, 5; 1996).

But Hazel O'Leary, the energy secretary, appeared to admit that the new procurements were intended to bolster the US industry. She said: "To remain competitive, we've got to maintain this dominance in computer capability." But she also said that "this was an open procurement — anyone could have bid". Department officials repeatedly refused to say how many companies had bid, or whether any of them were from outside the United States.

The new machine will be accessible to non-weapons scientists inside and outside Department of Energy laboratories. David Nowak, head of the ASCI programme at Livermore, says that a small-grant programme — worth \$4 million this year, growing to \$12 million by 1998 — will support university scientists who want to use it.

Colin Macilwain



by five orders of magnitude by 2010. The first machine bought under the programme was a 1.8-teraflop computer (1.8×10^{12} floating point operations per second) ordered from Intel last year by Sandia National Laboratory in New Mexico.

The Lawrence Livermore machine will deliver three teraflops, but is distinguished by its huge memory capacity. A ten-teraflop machine will be bought for Los Alamos National Laboratory in New Mexico, for operation by 2000, officials said, and thirty-teraflop and hundred-teraflop computers will follow by 2004.

Clinton used the announcement to boast about his administration's track record in science. America is competing with the rest

Cray files formal 'dumping' complaint

Washington. Cray Research, the supercomputer supplier, has formally filed complaints with the US Department of Commerce and the International Trade Commission (ITC) alleging that its rival NEC is "dumping" a supercomputer that the Japanese company hopes to supply to the National Center for Atmospheric Research (NCAR) in Boulder, Colorado.

NCAR has chosen NEC to supply four large vector supercomputers under a \$35-million, five-year contract. But Cray says the price is "substantially and illegally below the cost of production" of the system (see *Nature* 382, 5; 1996).

Announcing the complaint, Robert Ewald, president of Cray, said that NEC was charging NCAR one-tenth as much as it charged two Japanese customers for the same processor units last summer. NEC had "cut prices sharply in Europe and Canada over the last few years", he said. "Now they are bringing that strategy to the US, where Cray has

stronger recourse to the law".

The Cray complaint is causing a major headache for the National Science Foundation (NSF), which funds NCAR and must approve the bid. According to Ewald, it could take a year to assess the complaint, and in the meantime "it will be up to the funding agency if it wants to continue" with the purchase.

NSF officials say the department has 20 days to decide whether the complaint warrants a full investigation, and that the foundation would make no comment and take no action during that period. It has not yet received the NCAR procurement contract for approval, says one official.

NSF is also waiting to see if the final version of its funding bill contains language, inserted by the House of Representatives in its version of the bill, docking the salaries of NSF officials if the procurement proceeds and the commerce department subsequently rules that it did constitute 'dumping'. **C. M.**

Software testing blamed for Ariane failure

Paris. Inadequate computer-software testing has been officially identified as the cause of the explosion of the European Ariane-5 satellite launcher 37 seconds into its maiden flight on 4 June.

Jean-Marie Luton, director general of the European Space Agency (ESA), said at a press conference in Paris last week, held to present the findings an independent board of inquiry, that the mishap was due to ESA's failure to upgrade adequately the flight control and guidance systems used on board Ariane-4.

The mishap will delay the Ariane-5 programme by six months and increase its cost by two to four per cent — or FF900 million (US\$180 million) to FF1.5 billion. "We are all to blame," said Luton.

The loss in the explosion of the four scientific satellites that were to be launched by Ariane-5 has added ECU400 million (US\$307 million) to the cost of the accident, according to ESA's science director, Roger

Bonnet. The satellites were part of the Cluster mission to study over two years the relation between the Sun and the Earth's magnetic field.

Their destruction "represents the loss of much unpaid work by scientists", Bonnet said. A total of 200 scientists from Germany, France and Britain, the main movers behind Cluster, as well as researchers from the United States, Japan and Russia, worked on the programme. Bonnet said the prototype of the Cluster satellites will be refitted, "possibly to be launched by Ariane-5 on its next flight next spring". The new scientific mission has, aptly, been named Phoenix (see *Nature* 382, 102; 1996).

Bonnet stressed that Phoenix's findings would not be comparable to those planned

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Ariane-5: failure occurred 37 seconds after lift-off.

for Cluster. "Cluster was to fly in a pyramid configuration, whereas Phoenix will be one-dimensional." He added: "A study is under way for a new three-dimensional measurement mission but its objectives have not been defined. It will probably take place around 2000 but it is likely to be scaled down compared to the original programme."

Bonnet maintained that the loss of the satellites would not jeopardize ESA's relations with its partners in the international space research community. The US National Aeronautics and Space Administration (NASA), a major partner in the Cluster project, is in particular to remain involved in Phoenix and possibly in future projects, he said.

"Solidarity in this type of situation is *de rigueur*," he said. "We did not alter our relationship with NASA when the Hubble telescope ran into trouble."

But Bonnet is worried about the future of ESA's research programmes. "Ministers [of the countries which operate ESA] last year ordered a ten per cent reduction in science funding by 1998, and I don't see an end to this trend in the absence of political will for progress in space research," he says.

Whatever cuts may be in store for European space scientists (see box, left), there is little doubt that Ariane-5 will be maintained, as it is an essentially commercial project. The inquiry board, headed by Professor Jacques-Louis Lions of the French Academy of Science, made 14 recommendations for the future of the launcher.

Among them are recommendations for better preparation and testing of equipment, for the revision of all flight software and for inclusion of "external participants when reviewing specifications, code and justification documents".

The board concludes by recommending "a more transparent organization of the cooperation among the partners in the programme". Luton predicted that "this will be the hardest recommendation to implement". But he promised to follow all the board's advice.

Roni Amelan

Budget cuts hit German funding for ESA

Munich. Germany's financial contribution to the European Space Agency (ESA) seems destined to fall significantly next year, in line with the reductions being planned in its support to five international research laboratories (see *Nature* 382, 285; 1996).

As part of its federal budget announced last month, the German research ministry proposed a cut of DM100 million (US\$68 million) in the budget of the German space agency, DARA. It is also proposing further savings by merging DARA with the national air and space research centre, Deutsche Forschungsanstalt für Luft- und Raumfahrt (DLR).

The space agency has a total budget of DM1.35 billion, DM1 billion of which is designated for ESA. If the cut is approved by parliament this autumn — as seems likely — Germany's contribution to ESA will be reduced by at least 7.4 per cent. This figure could be even higher if Germany decides to protect its national space programmes. Christian Lenzer, science spokesman for the ruling Christian Democrat party, says that his party supports such a move.

Decisions have yet to be made on the precise division of the cuts between national and international space programmes. But Gernot Hartmann, DARA's director of science programmes, says that he believes the current ratio of spending is likely to be maintained. He says commitments to ESA are expected to be honoured. "The cuts will probably mean that we will not be able to start

any new ESA projects," he says.

Jürgen Rüttgers, the German research minister, has given the directors of DARA and DLR until October to make suggestions on how their proposed merger could be made most effective.

DARA was set up in 1989 to coordinate the space activities of several ministries. But in practice it has concentrated almost exclusively on the interests of the research ministry. Other ministries with interests in space, such as telecommunications, transport and defence, have not been prepared to hand over responsibility. So the agency's staff of 270 has ended up handling a relatively small budget.

Jan-Baldem Mennicken, director general of DARA, says that any merger proposal that DARA and DLR agree will avoid a conflict of interest between management of space programmes and the research and development activities of DLR, which some scientists fear. Hartmann also says relations with ESA should remain unchanged.

The opposition Social Democrats have criticized the merger, saying that it will adversely affect the continuity of space science policy. Instead, the party argues, other ministries should be forced to transfer their space programmes to DARA, as had been agreed earlier this summer by parliament. But given the pressures on the German budget, many scientists are not surprised by the sudden squeeze on space science.

Alison Abbott & Quirin Schiermeier

CERN comes under fresh financial pressure

London. Completion of the Large Hadron Collider (LHC) at the European Laboratory for Particle Physics (CERN) in Geneva, Switzerland, may have to be delayed, following a move by Germany to reduce its contribution to the laboratory next year by almost 10 per cent.

The proposed reduction, approved by the German cabinet three weeks ago (see *Nature* 382, 285; 1996), has yet to be officially approved by parliament. Nor is it clear how it is likely to be implemented, given that the cut would appear to conflict with previous German commitments to support CERN's budget at its current level.

Although such cuts can be agreed only by CERN's council, Germany and the United Kingdom, already facing difficulties with CERN's annual subscription, could force them through, given their voting power on the council. But other member states are also increasingly concerned about the burden of the annual subscription.

One result could be increased pressure from some member states to persuade the United States and Japan to join as full members, a position with which British officials, for example, say that they have little difficulty.

But other countries would strongly resist such pressure. "CERN is a European facility, and its rules are not compatible with the admission of full members from outside Europe," says Hubert Curien, the former French research minister who is chairman of the laboratory's governing council.

Any such change, he says, would require revision of the treaty under which CERN was established in 1954. A more likely outcome would therefore be a decision to delay the completion of the accelerator.

The planned cut in Germany's CERN contribution next year is expected to be followed by similar reductions in its contribution for the three subsequent years. It comes as part of a package of public spending reductions that has been introduced by the coalition government of Chancellor Helmut Kohl to meet the terms of eventual membership of a single European currency.

The German research ministry (BMBF) last week corrected initial figures it had provided on the size of the reduction, and said that the country's contribution to CERN is in fact intended to fall from DM265.7 million (US\$178 million) this year to DM240.9 million next year. This represents a cut in constant funds of 9.3 per cent, and in real terms of almost 11 per cent, once inflation is taken into account.

CERN officials are declining to comment on the German proposals, pointing out that they have not received official confirmation in writing from Bonn.

Christopher Llewellyn Smith, the director-general of CERN, says that the labora-

tory is already having to work with a declining budget, as spending is due to increase more slowly than the anticipated rate of inflation. He points out that recently introduced economies have led to cuts in staff and a saving from other projects of SFr25 million (US\$21 million) a year.

But if the additional proposed German cut is confirmed, it will inevitably be a blow to plans for the construction of the LHC, approval of which was obtained from member states in 1994 only after budgetary concessions to Germany and the United Kingdom (see *Nature* 372, 713; 1996).

The treaty under which CERN operates fixes the level of Germany's annual subscription as a fraction of the laboratory's overall budget. One concern is that the obvious way

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Helping hands: Japan's science minister Kaoru Yosano (left) and Llewellyn Smith of CERN paint a doll last year to wish the LHC good luck.

to implement a cut in this contribution would be to reduce the total budget — and hence contributions by other members — by the same proportion.

Two strategies could reduce the annual costs to CERN members of constructing the LHC. One would be to seek to make the United States and Japan full members of what would become a 'global' laboratory, requiring both to increase their payments to the basic cost of the accelerator. This is seen as unlikely in current political circumstances.

The other possibility would be to delay the planned date of completion. At present, the collider, which has been designed to explore the predicted characteristics of the Higgs boson, is planned to come on line at two-thirds of its final energy in 2004, and to reach full energy in 2008.

If the United States and Japan agree to provide the additional funding that is currently under negotiation, CERN officials had been hoping to bring the date for reaching full energy forward to 2005. A reduced European contribution could result in this date slipping back, although officials argue that any annual savings gained by pursuing such a strategy would be minor, while the overall construction costs of the accelerator would inevitably increase.

Such a delay would also have repercussions for non-European involvement. Although foreign physicists would still be keen to participate, their political paymasters might be tempted to look to other projects to fund.

"We would be very disappointed if the project were to be put back," says Sidney Drell, deputy director of the Stanford Linear Collider, and chairman of a panel of physicists which has drawn up plans for the Department of Energy for possible US involvement in the LHC.

Yet any move to reduce the annual costs of the collider to CERN's member states is likely to be welcomed in Europe's capitals, as most governments are experiencing tight squeezes on public spending.

Britain's science minister, Ian Taylor, for example, has already given Germany's announcement a tacit welcome as reflecting and reinforcing UK pressure on CERN to reduce costs to free more money for Britain's domestic particle physics programme (which includes constructing the detectors with which collider experiments will be conducted).

France and Italy are facing similar difficulties in funding their domestic research efforts because of their commitment to CERN, and both are generally thought likely to react sympathetically to any move to reduce the pressure of the CERN subscription on their domestic research budget.

"France remains committed to CERN in general, and to the LHC in particular," says Claude D  traz, director of the National Institute for Nuclear and Particle Physics. "But CERN should open its books for a thorough review of what could be done in the present political situation. We cannot keep our eyes closed to the difficulties that we all face."

At the very least, the German move is likely to bring forward a review of the funding prospects for the LHC. It was agreed in 1994 that such a review should take place next year. Curien says: "We may ask the management of CERN to be ready as soon as possible to make this review."

Indeed, some government officials feel that the implications of British and German demands for cost reductions are sufficiently serious to warrant a meeting of the research ministers of member states within the relatively near future.

In Germany itself, the Deutsches Elektronen-Synchrotron (DESY) in Hamburg has, in contrast to CERN, been promised level funding for next year. Officials in the research ministry defend this decision on the grounds that while CERN, despite its financial difficulties, is still able to find money for new scientific experiments, they argue that this has recently become virtually impossible at DESY.

David Dickson

Discovery of error brings sea-rise estimates back into line

Washington. A data-processing error has led to scientists working with data from a US altimeter on the Topex/Poseidon oceanography satellite to lower their estimates of global sea-level rise.

Preliminary findings based on Topex data in 1994 suggested that sea level was rising 3 millimetres annually, a figure that grew to 5 mm as more information came in. But scientists had been suspicious, as these figures did not agree with data from the French Poseidon altimeter on the same satellite, nor with Earth-based tide gauge measurements. After French space agency engineers found a mistake in an algorithm used to correct readings from a clock on board the US spacecraft, the Topex estimate dropped to between 1 and 3 mm per year, in line with the other measurements. □

UK research units under scrutiny

London. The British government is to take a close look at six research units supported by the Medical Research Council (MRC), to see whether they should be 'privatized', Ian Taylor, the science and technology minister, announced last week. The MRC's Dunn Nutrition Unit, Virology Unit, Toxicology Unit, Reproductive Biology Unit, Radiation and Genome Stability Unit, and Mammalian Genetics Unit all face "prior option reviews", along with other public sector research establishments. □

Japanese task force for *E. coli*

Tokyo. Japan's prime minister last week set up a Cabinet-level task force to deal with the growing epidemic of food poisoning caused by the 0157 strain of *Escherichia coli*. The epidemic has struck more

than 8,000 schoolchildren in Japan and caused eight deaths. The task force includes 14 ministers, including those for health, education, agriculture, home affairs, labour, finance and transport.

They will order meat-processing facilities, often the primary source of infection, to check sanitation, and produce a checklist for improving the hygiene of school lunches. Debate is raging about the best way to treat those infected. Many doctors are prescribing antibiotics. But some argue that this may trigger bacteria to release toxins, causing renal failure and brain haemorrhaging. □

Soyuz launches — into market

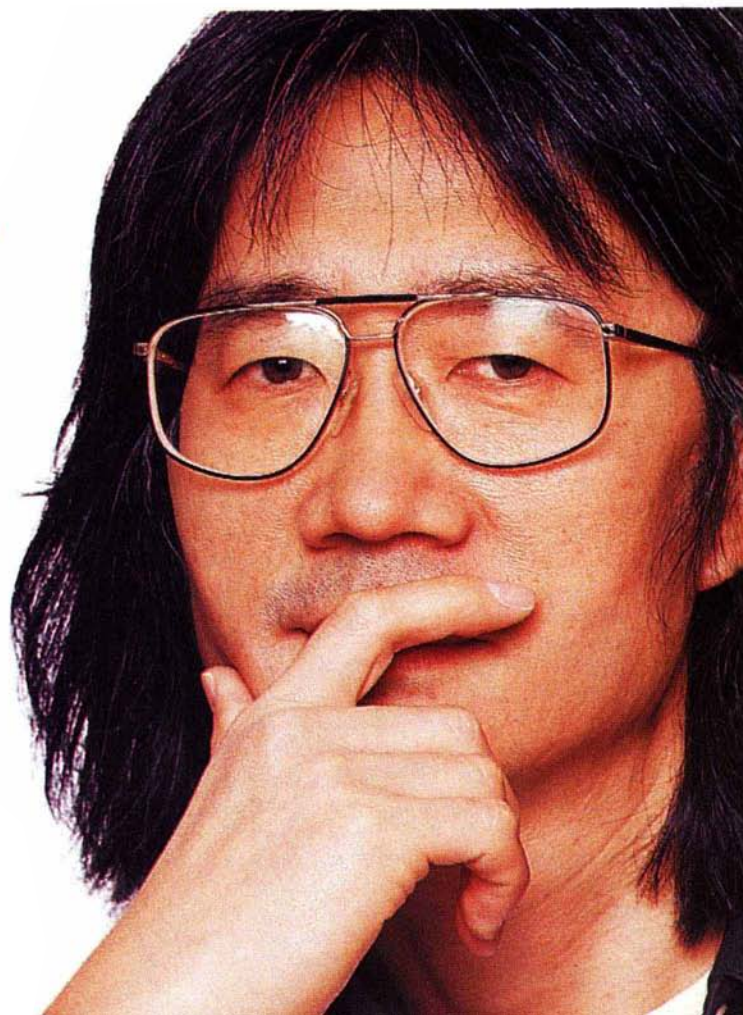
London. Russian and European space launcher companies are joining forces to market the Soyuz family of launch vehicles around the world. The Russian bodies concerned are RKA, the agency which oversees Russian space activities, and the Samara Space Centre, which is in charge of developing, building and launching a range of rockets in addition to the Soyuz.

The European companies are France's Aerospatiale, the space vehicle manufacturer, and Arianespace, which has been responsible for the Ariane launcher developed by the European Space Agency. By combining their expertise and pooling finances, the four companies hope to encourage international interest in the Soyuz system which, they claim, is one of the world's most reliable rockets. □

Clinton nominates science board

Washington. President Bill Clinton has nominated five new members to the National Science Board, the governing body of the National Science Foundation. They are John Armstrong, former vice-president for science and technology at IBM; M. R. C. Greenwood, formerly of the White House science staff and now chancellor of the University of California at Santa Cruz; Stanley Jaskolski of Eaton Corporation, the electrical engineering company based in

To **save an hour**
each time you
purify histidine-
tagged proteins,
put it on the
tip of a syringe



Cleveland, Ohio; Vera Robin, an astronomer at the Carnegie Institution of Washington DC; and Bob Suzuki, an engineer who is president of California Polytechnic University, Pomona. The nominations have to be confirmed by the Senate. □

UK companies lag competitors

London. A study commissioned by the British Treasury from the Science Policy Research Unit at the University of Sussex has found that while publicly-funded research can yield significant economic benefits, many UK companies are not taking adequate advantage of it, unlike their competitors in the United States and Japan.

The report admits that measuring value for money is not easy. But it says that basic research "seems to have had a substantial impact on productivity", in particular by supporting a network of experts with economically valuable skills, and supplying new methodologies, instrumentation and information. Britain's chemicals and pharmaceuticals sectors in particular have been able to use this information to develop a strong research base, it says. □

Tokamak link for remote access

Tokyo. The Japan Atomic Energy Research Institute (JAERI) has established a high-capacity computer link with Los Alamos National Laboratory in New Mexico and the Princeton Plasma Physics Laboratory in New Jersey. This will allow US researchers to carry out and observe experiments remotely in real time on the JT-60 Tokamak fusion device facility, which JAERI runs in Naka Machi, north-east of Tokyo.

A separate ISDN link allows video conferencing between the United States and Japan. JAERI hopes to extend the network to Europe in the near future. The link is seen as a model for remote access to the proposed International Thermonuclear Experimental Reactor (ITER), which Japan is lobbying hard to host. □

Delaney cancer clause killed off

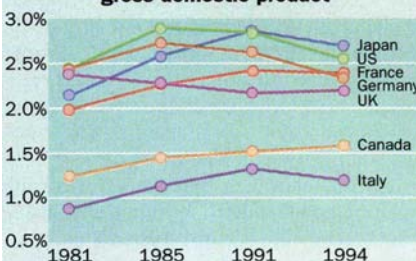
Washington. Both houses of the US Congress have unanimously passed a pesticide regulation bill that kills the Delaney clause, a notoriously unenforceable rule which banned from processed foods any chemical found to cause cancer — at however small a dose. The bill, which President Bill Clinton is expected to sign shortly, will replace the 1958 clause with one requiring "reasonable certainty" that the chemical will do no harm. Remarkably, the bill won the support of environmental groups and the food industry. The two sides had reportedly grown tired of decades of fighting over the issue. □

R&D spending decline continues

Paris. The decline in spending on research and development by most Western nations is continuing, according to the latest figures from the Organisation for Economic Cooperation and Development (OECD). In its latest *Main Science and Technology Indicators*, published last month in Paris, the OECD points out that the slowdown in spending on R&D as a proportion of Gross National Product that had been observed in 1993 was "significantly less" in 1994 (see figure). But provisional data indicate this stability "will not continue into 1995".

The deterioration in science activity since 1994 in Japan is indicated by falling R&D spending, and in the fact that its inventors are applying for fewer patents abroad. □

Gross domestic expenditure on research and development as a percentage of gross domestic product



HisTrap kit: saving an hour couldn't be simpler

Would you like to save one hour in histidine-tagged protein purification?
To load, purify and elute in 15 minutes? Now it's possible—time after time.

The HisTrap™ Kit presents you with a solution so simple it works with a syringe. Inside the kit is all you'll need for up to ten affinity purification runs: pre-packed cartridges, ready-made stock solutions, a protocol that's easy to follow, and, of course, a syringe.

You'll get high purity recombinant proteins as the kit lets you use the well-known binding of poly(histidine) sequences to immobilized nickel. What's more, it saves you the effort of weighing out buffer salts.

Molecular biologists have been purifying recombinant proteins with Pharmacia Biotech media from the very beginning. Call us at 1 (800) 526 3593 from the USA, +81 (0)3 3492 6949 from Japan or +46 (0)18 16 50 11 from Europe and the rest of the world (or meet us on the Internet at <http://www.biotech.pharmacia.se>). Ask about the kit that saves an hour.

Pharmacia Biotech
Uppsala, Sweden. (And the rest of the world)

A column from the HisTrap™ Kit, magnified 280%.



"Purity, simplicity and time savings: a product that gives you all these, lets you take a break from the lab for a bagel, coffee and...hmm, maybe a haircut?" Kyungsun Suh, Ph.D., at the Rockefeller University, New York, NY

Ozone layer: the road not taken Counterattack

SIR — As distinguished and experienced participants in the evolution of scientific understanding of ozone-layer depletion, Prather *et al.* (*Nature* **381**, 551–554; 1996) provide a valuable quantitative perspective on the advantages of acting early to correct global atmospheric catastrophe. Although scientists have given an early warning on climate change, there has been little success so far in slowing the growth in greenhouse gas emissions. It is therefore worth looking a little more deeply at some of the reasons why protection of the ozone layer was so successful.

The 1996 phase-out of chlorofluorocarbons (CFCs) and other ozone-depleting substances (ODSs) under the Montreal Protocol is economically and environmentally successful for four reasons. First, scientists ultimately persuaded industry of the need for prompt action. Second, governments selected flexible, market-based regulation. Third, industry cooperated to speed development and selection of new technology. Finally, industry helped policy-makers to choose schedules that were technically feasible and allowed time for wise choice.

In retrospect, it can be seen that more science, earlier, could have given even greater warning that CFCs destroy the ozone layer. This would have reduced dependence on ODSs and saved the cost of eliminating them. The importance of science in protecting the ozone layer is strong justification for continued financing of basic atmospheric research. If science had been less well funded, scientists less proactive or diplomats less successful, signing of the protocol might have been delayed. Moreover, the cost of the ODS phase-out would have been about half as much if CFC manufacturers had been less successful in their political interventions between 1974 and 1987. Much of the chlorine in the atmosphere is from applications such as cosmetic products for which there were technically and economically feasible alternatives a decade before the protocol was signed and two decades before the ODS phase-out.

Prather *et al.* hypothesize on what might have happened if the phase-out had started later but, to protect the ozone layer, industry had had to achieve the same phase-out schedule. With a global emergency, elimination of many ODS applications with identified alternatives could have been significantly accelerated with small increases in ultimate costs. Such uses include aerosol

products, metal cleaning, rigid-foam building insulation, flexible foam and many halon applications. In some cases, alternatives and substitutes were quickly identified and could have been more quickly implemented under emergency conditions. Such uses include HFC-134a for automobile air-conditioning and refrigeration; hydrochlorofluorocarbon (HCFC) substitutes for refrigeration and air-conditioning, sterilization and rigid foams; and aqueous cleaning of electronic assemblies. In other cases, five years or more of well-funded, aggressive efforts were required to identify new technology for critical applications that had no alternatives and substitutes in 1987. If work on alternatives had not begun in 1987, ODS use would have had to continue after 1996. Such uses include aerospace applications such as electrical, optical and precision cleaning of both civilian aircraft and military weapons.

The most serious consequence of a later start on ozone-layer protection would probably have been to owners of automobiles, refrigeration equipment and air-conditioners which now depend on recycled CFCs. Recycled CFCs are inexpensive, and retrofit kits are just entering the market for some equipment.

A late start would also have required industry and consumers to select from among the very first technologies available. Some of the most environmentally acceptable, cost-effective substitutes were second-generation replacements for CFCs. Much CFC-113 solvent use in electronics manufacturing would have been replaced with HCFCs, chlorinated solvents and alcohols instead of technically and environmentally superior no-clean soldering. Moreover, industry is less likely to finance continuing research and development once a technology is selected.

The protocol phase-out schedule has maintained public confidence in the value of the changes that are being made; a later start might have been too expensive to consumers and some companies to maintain necessary political support. Even now, there is concern that developed countries will fail to meet their obligations for financing the phase-out in developing countries.

In sum, the Montreal Protocol was none too early, hopefully just in time, but possibly a little too late.

Stephen O. Andersen

Stratospheric Protection Division,
Environmental Protection Agency,
401 M Street SW,
Washington, DC 20460, USA

Alan Miller

Center for Global Change,
University of Maryland,
College Park, Maryland 20740, USA

SIR — In his review of *Confronting the Experts* (*Nature* **382**, 35–36; 1996), Walter Gratzer selected my chapter for the harshest criticism. The reason he gave was his assertion that I believe “for instance that all attempts at cellular fractionation generate nothing but artefacts, that practically everything revealed by the electron microscope is an artefact of fixation and staining, that there is no such thing as a lipid bilayer membrane, that the known cytoplasmic granules are mirages, the nervous system tissue consists of granular ‘ground substance’ with ‘naked nuclei’...”.

Each of these five statements as quoted is such a gross distortion of my views that I can only conclude that Gratzer has not read any of my original publications on these topics. I might have expected that a senior colleague would have read the evidence upon which my conclusions are based before condemning them.

In passing, he remarks that my finding that ATP is hydrolysable by visible light could not be true because it does not absorb. Perhaps he does not know that colourless solutions of adrenaline, lactate, fumarate, hydrogen peroxide, scintillation fluids — and, yes, ATP — are stored in dark bottles because they are unstable in visible light.

Harold Hillman

Unity Laboratory of Applied Neurobiology,
76 Epsom Road,
Guildford, Surrey GU1 2BX, UK

Manners please

SIR — Why are academics rude to one another? Discussion of opposing views so often degenerates into name-calling. An investigator new to a field will make many personal discoveries, some of which were learnt in kindergarten by the expert. The expert, however, should not launch a personal attack on the novice for verbalizing such ideas. Indeed, it is always possible that a fresh mind, with a new perspective on a problem, will make suggestions previously overlooked. Criticism aimed at the person rather than the issue in hand serves no good purpose. It is a poor reflection on the perpetrator, since it indicates frustration and an inability to make further constructive comment. Harsh words generate alienation and discouragement. We all make mistakes from time to time, so a sympathetic attitude would be of universal benefit.

Valerie Good

Centre for Mechanisms of Human Toxicity,
University of Leicester,
Hodgkin Building,
Lancaster Road,
Leicester, LE2 2AB, UK

corres@nature.com

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only, or e-mailed to corres@nature.com

Bureaucracy defeated at EPA

SIR — Dr David Lewis's Commentary article on the US Environmental Protection Agency (EPA)'s science (*Nature* **381**, 731; 1996) shows him to be one of the dedicated scientists whose contributions to protecting the environment have been the hallmark of EPA's research and development programme. His frustration and concern clearly come through. However, from my view as an informed outsider who formerly headed EPA's Office of Research and Development (ORD), his Commentary may be misleading as to the direction of changes in recent years.

The current head of ORD, Robert Huggett, is an internationally known environmental scientist who has worked very effectively to deal with the many problems inherent in US bureaucracies, and particularly in those whose regulatory power attracts attention. Bureaucratic brick walls I left behind at EPA a decade ago have crumbled, or ways have been found to drive around them. Under Huggett's leadership, and that of his immediate predecessor Erich Bretthauer, there has been a needed increase in funding for ecological research and a marked improvement in administrative approaches to selecting and promoting scientists and engineers. Peer review and quality assurance have extended ever more deeply into EPA, now even reaching some

regulatory offices. The strategic planning process has improved greatly, and by clearly articulating the relationship between EPA's regulatory priorities and its scientific and technical research, Huggett has violated the bureaucrat's rule of avoiding accountability.

By achieving an increase in emphasis on the extramural programme, Huggett and the EPA Administrator, Carol Browner, have enlisted the external scientific community to an amazing extent — they received more than a thousand grant proposals last year. Unfortunately, because of inadequate funding, they could support only a handful of these. The true test of this strategy will come this year. To be successful rather than counterproductive, the extramural programme must be able to obtain further direct dollar support and also to divert funding from noncompetitive private contractors to the competitive peer process. As Lewis correctly points out, the decrease in extramural funding for cooperative agreements to work with university scientists has not been helpful to EPA's intramural laboratory efforts. EPA and its laboratories need to be able to resist the congressional pressure that has forced it to continue laboratory spending on private contractors rather than cooperative agreements and grants.

Lewis is certainly correct in pointing out the frustrations of dealing with heavy-

handed bureaucratic rules that may be appropriate, if at all, to ensure that the taxpayer gets the highest quality at the lowest price for the purchase of tanks or of toilet paper. My impression, however, is that this negative phenomenon of the early 1990s has eased in the past few years, in part because of principled resistance by EPA scientists who have won their cases, although not without great cost to them and to their colleagues. The success of the present EPA leadership in decreasing the burden on scientist-administrators is supported by the decrease in new directives since its peak in 1993. The continuing reorganization of ORD is likely to continue the increase in the ratio of scientists to administrators.

There is no disagreement with Lewis's metaphor about building a scientifically sound foundation for environmental protection. But he is in error on the issue of building the actual foundation for a new laboratory complex in Research Triangle Park, North Carolina. In 1985 I failed to convince the Office of Management and Budget of the need for a new centralized research building in North Carolina to replace inadequate and expensive leased sites. It is good news that EPA leadership has finally accomplished this feat.

Bernard D. Goldstein

*Environmental and Occupational Health Sciences Institute,
PO Box 1179,
Piscataway, New Jersey 08855-1179, USA*

Hot biology papers

SIR — An analysis of highly cited ('hot') papers in biology, published mainly in *Nature*, *Science* and *Cell*, reveals that the selected hot papers have more authors, more institutions and more funding sources than other papers. For the study, I analysed 16 issues, 71 papers identified by *Science Watch* between 1993 and 1995. I excluded six hot papers with 20 or more authors. I compared 65 hot papers with 414 papers in 28 issues of the three leading journals that contained at least one hot paper.

My analysis reveals that the mean number of authors per hot paper is 6.54 for

Nature, 6.62 for *Cell* and 7.71 for *Science*, whereas the mean number of authors per paper varies between 4.36 and 4.66. There seems to be a distinct trend towards more authors as compared to earlier findings¹.

The mean number of institutions per paper is defined as papers listing more than one institution participating in the research. The table shows that the mean number of institutions per paper is 2.06 for *Science*, 1.91 for *Nature* and 1.72 for *Cell*. The proportion of multi-institutional papers is higher in the hot papers than in the rest.

Funding is known to be associated with multiple authorship²⁻⁴. My data show that there is considerable variation in the mean number of funding sources for two different

groups. When I analysed the data according to the funding sources, I found that papers reporting four or more funding sources have more authors (for example, in *Science*, 7.7 authors and 8.6 authors in hot papers compared with 4.4 and 6.0 authors for the rest).

Zhang Haiqi

*National Institute for the Control of Pharmaceutical and Biological Products,
Beijing 100050, China*

1. Satyanarayana, K. & Ratnakar, K. V. *Scientometrics* **17**, 363–371 (1989).
2. Price, D. J. de S. *Science* **212**, 986 (1981).
3. Pao, M. L. *Inform. Process. Manag.* **28**, 99–109 (1992).
4. Mussarakis, S. *Am. J. Roentgenol.* **163**, 973–979 (1994).

Present address: Medical Information Center for Education and Research, Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105, Japan.

DISTRIBUTION OF PAPERS BY THE MEAN NUMBER OF AUTHORS, INSTITUTIONS AND FUNDING SOURCES

Periodicals	Papers	Authors	Mean no. of authors	Institutions	Multi-institutional papers (%)	Mean no. of institutions	Funding sources	Funded papers (%)	Mean no. of funding sources
<i>Nature</i>									
Hot papers in biology	24	157	6.54	54	79.17	2.25	78	83.34	3.25
Articles/Letters to Nature	152	663	4.36	291	59.48	1.91	295	75.66	1.94
<i>Cell</i>									
Hot papers in biology	21	139	6.62	45	66.67	2.14	72	90.47	3.43
Articles	139	632	4.55	225	44.61	1.62	381	97.12	2.74
<i>Science</i>									
Hot papers in biology	14	108	7.71	46	85.71	3.28	53	92.86	3.78
Research articles/Reports	123	542	4.41	254	58.06	2.06	290	83.74	2.35
<i>PNAS</i> (US) etc.									
Hot papers in biology	6	28	4.66	9		1.50	18		3.00

Genetic testing and insurance

SIR — Now that the US Senate and House of Representatives have denied the US health-insurance industry the right to use human genetic information to disqualify applicants¹, this rule needs to be applied worldwide. Nickerson² has already called for a uniform policy in relation to testing; otherwise, as he points out, a two-tier health-insurance system could well develop internationally. This could siphon off those who are 'normal' on the basis of the genetic tests applied, leaving the untested remainder to pay higher premiums.

The Helsinki Declaration, as revised at the 29th World Medical Assembly in Tokyo in 1975, states in Section iii 4 that "[i]n research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject"³. It is precisely these considerations of the well-being of the subject that would be placed at risk, in the name of society, were genetic testing on medical grounds to result in disclosure to insurance companies. Disclosure would lead, as has often been pointed out, in many cases to changes in a patient's well-being, such as his or her insurance rating, or uninsurability, employability, and the peace of mind of the patient and other family members. Even if a patient chooses not to know the results of a test, these would be revealed by the size of premiums or lack of insurance. Moreover, with respect to testing, "the benefits of their participation might not be realised by them or their offspring, but might help future generations"⁴.

To add the penalties outlined above for genetic discoveries arising from tests would be detrimental not only to a patient's well-being but also to his or her perception of testing. It could be argued that to reveal the results of genetic tests to insurers might allow one sector of society (the insurance industry) to draw financial benefits against the best interests not only of the individual patient but also of research and society in general. Many factors, for example, deter women from being tested for the breast cancer gene *BRCA1*. Geller *et al.*⁵ warn that interest in testing is distinct from participation in testing; and they stress the need for sensitivity in relation to the general distrust of health services by certain sections of the population, and the 'slippery slope' from the perceived social responsibility to be tested to advance medical science to coercion. Also, in some cases, there might be coercion to be tested for a patient to stand a chance of health insurance, if

disclosure was not ruled out. It is important, therefore, that members of the medical profession should retain, and be known to retain, complete confidentiality with regard to genetic test results. It is also important that they alone carry out genetic testing in order that the well-being of their patients may be protected.

Richard J. Skaer

Peterhouse,

Cambridge CB2 1RD, UK

Climate debate

SIR — It is regrettable that you urge "political support for abatement strategies" before a scientific controversy is settled¹.

Although you are dismissive of those who are critical of the Intergovernmental Panel on Climate Change (IPCC), your leading article nevertheless makes three things quite clear.

(1) A crucial chapter of the IPCC's report was altered between the time of its formal acceptance and its printing.

(2) Whether in accordance with IPCC rules or not — still a hotly debated matter — "there is some evidence that the revision process did result in a subtle shift ... that ... tended to favour arguments that aligned with the report's broad conclusions". (Critics of the IPCC would have used much stronger words.) The leading article further admits that phrases that might have been (mis)interpreted as undermining these conclusions "disappeared" in the revision process.

(3) Unnamed "IPCC officials" now claim that the reason for the revisions to the chapter was "to ensure that it conformed to a 'policymakers' summary' of the full report...". Their claim begs the obvious question: should not a summary conform to the underlying scientific report rather than vice versa?

The IPCC summary has many problems of selective presentation of facts², not the least of which is that it totally ignores global temperature data gathered by weather satellites, which contradict the results of models used to predict a substantial future warming. It seems to me that IPCC officials, having failed to validate the current climate models, are now desperately grasping at straws to buttress the (rather weak) conclusion that "the balance of evidence suggests a discernible human influence on global climate". In this crusade to provide a scientific cover for political action at the Global Climate Treaty negotiations in July in Geneva, they (mis)used the work of respected scientists who never made such extravagant claims^{3,4}.

The scientific response to these recently published papers has not yet appeared.

Indeed, some papers quoted in support of the IPCC conclusion had only been submitted for publication and were still in preprint form when the IPCC report was written.

The leading article correctly observes that "the integrity of the reviewing and approval process is ... an essential element in assuring the credibility of the resulting conclusions". We should not be pushed into adopting hasty policies before journals such as *Nature* print the scientific responses.

S. Fred Singer

Science & Environmental Policy
Project,

4084 University Drive, Suite 101,
Fairfax, Virginia 22030-6812, USA

1. *Nature* **381**, 539 (1996).

2. Singer, S. F. *Science* **271**, 581 (1996).

3. Mitchell, J. F. B. *et al. Nature* **341**, 132-134 (1995).

4. Santer, B. D. *et al. Clim. Dyn.* **2**, 79-100 (1995).

Not Mussolini but Volterra

SIR — Alison Abbott credits Benito Mussolini (*Nature* **381**, 720; 1996) with establishing Italy's National Research Council (CNR) after the First World War. Mussolini was prime minister at the time, but the driving force was the distinguished Italian statesman of science and mathematician Vito Volterra. When Mussolini became Italy's dictator in 1925, all state institutions, including the CNR, became fascist bureaucracies.

There is no telling what the CNR might have become if Volterra, an ardent anti-fascist, had been allowed to develop it. The custom of filling university positions through national competitions is embedded in the formation of modern Italy. Luigi Berlinguer has his work cut out.

Judith R. Goodstein

University Archives,
California Institute of Technology,
Pasadena, California 91125, USA

Dropping bricks

SIR — Once again I write to you to ask you to be a little more careful with your Greek. In the review of *Tectonics* by Moore and Twiss (*Nature* **381**, 570; 1996), the reviewer says that "the ancient Greek τεχτονική means art of building". This is not so. He has confused two things: τεκτονική, which does indeed mean the art of building, is spelt with a *kappa* and is the root of the word 'tectonics', and τεχνική, which means skilful or workmanlike, is spelt with a *chi* and is related to the root of words such as technology (and others also spelt with a *ch* for *chi* rather than a *c* for *kappa*).

Anna Hodson

Science Museum,
London SW7 2DD, UK

1. *Nature* **381**, 10 (1996).

2. Nickerson, P. H. *Nature* **380**, 386 (1996).

3. Declaration of Helsinki — 1964 and 1975

URL: <http://cme-mac4.bsd.uchicago.edu/CCMEPolicies/MedCodes/helsinki>

4. Parker, L. S. *Trends Genet.* **11**, 521-523 (1995).

5. Geller, G. *et al. Nature Genet.* **11**, 364 (1995).

A Nobel tale of wartime injustice

Elisabeth Crawford, Ruth Lewin Sime and Mark Walker

Recently released documents give the inside story of Otto Hahn's 1944 Nobel prize in chemistry for the discovery of nuclear fission. They reveal flaws in the award-making process — and an attempt to rewrite history.

MANY historians think that an injustice was done in 1945 when Otto Hahn was awarded the 1944 Nobel prize in chemistry for the discovery of nuclear fission. From the beginning the award was controversial and seemed unfair because Hahn's two colleagues, the physicist Lise Meitner and the chemist Fritz Strassmann, did not share in the prize¹.

The Royal Swedish Academy of Sciences' official records of the Nobel prize deliberations of 1945 were released to scholars after the usual 50-year delay. These documents, together with records from earlier years and private correspondence², shed light on why Hahn was honoured and Meitner and Strassmann were not. Although 1945 was the year of the final decision, it marked the end of a long process that began when nuclear fission was first announced in 1939. Taken together, the documents do not present a complete picture, but they do reveal flaws in the Nobel decision-making process. They show the difficulty of evaluating an interdisciplinary discovery, and a lack of scientific expertise in theoretical physics. And they shed light on Sweden's scientific and political isolation during the Second World War, which hindered understanding of Meitner's contributions to the discovery.

The discovery of nuclear fission, late in 1938, was the end-result of a complex investigation pursued by physicists and chemists across the world. The story began in 1934 in Rome when Enrico Fermi first irradiated uranium with neutrons, detected several new radioactive species and suggested that transuranic elements had been produced. In Berlin, Meitner, Hahn and Strassmann formed an interdisciplinary team that relied on analytical chemistry and radiochemistry for assigning the activities to specific elements and isotopes and on nuclear physics for measuring and interpreting the reaction parameters and mechanisms. Meitner, who was of Jewish origin, fled Germany in July 1938 and took a position in Stockholm.

Their collaboration continued, however, through Meitner's regular correspondence with Hahn and in their crucial meeting in Copenhagen in November

1938, when she objected to the most recent findings and urged him to verify them. A few weeks later, Hahn and Strassmann identified barium among the uranium products — evidence that the nucleus had split. Meitner was informed and, with her nephew, the physicist Otto Frisch, provided the first theoretical interpretation of the fission process.

In Nazi Germany, however, joint publication was not an option. Hahn and

committees evaluate only scientists who have been nominated. Nominations may be made by Swedish and foreign members of the academy, members of the committees for physics and chemistry, Nobel laureates in physics and chemistry, and invited scientists. The nominating period ends on 1 February. A select group of candidates is examined in special reports prepared by a committee member. These reports are discussed in the committees and sum-

Hulton Getty

marized in a general report, which ends with a recommendation. The special and general reports are the basis for the academy's decision, which must be made before 15 November.

Over the years, the committees' work had come to reflect two principles: precedence — each new decision should reflect the accumulated wisdom of previous ones; and consensus — all recommendations should have the full support of the committee. Both principles came into play in the case of Hahn, Meitner and Strassmann. Radioactivity and radioactive elements had traditionally been subjects in the domain of the chemistry committee³. Until 1945 nuclear fission was always evaluated in this committee — even though it was discussed in the physics committee — with important consequences.

Both Hahn and Meitner had been nominated repeatedly for Nobel prizes, but they had never been recommended for an award.

The discovery of nuclear fission in December 1938 changed all that. By 1 February, the deadline for the 1939 prizes, Theodor Svedberg, chairman of the chemistry committee, proposed an undivided prize for Hahn. Svedberg ignored Strassmann, probably because he was the junior member of the team, but did suggest that the prize might be divided between Hahn and Meitner because of their prior collaboration.

In his report, Svedberg mentioned the article in *Nature* in which Meitner and Frisch gave the theoretical explanation for fission, but he apparently misunderstood it or did not read it carefully. Meitner and Frisch had used the liquid-drop model developed by Niels Bohr and others as the basis for their theoretical explanation. But

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Otto Hahn: winner of the 1944 Nobel prize in chemistry.

Strassmann published the barium finding in *Naturwissenschaften* in January 1939; Meitner and Frisch's theoretical note appeared in *Nature* the following month. These separate reports seemed to split the discovery between chemistry and physics, experiment and theory, and Germans and émigrés. Rather than reflecting the science, which remained interdisciplinary to the end, the divided reports were the result of Meitner's forced emigration and the political oppression of the time.

Nobel prizes in the physical sciences are awarded through a three-stage process involving the committees for physics and chemistry, the relevant sections of the academy and, finally, a meeting of the entire academy. The five-member com-

Svedberg noted only that Bohr's liquid-drop model had been used, without mentioning that Meitner and Frisch had used it. Indeed, as proof that Bohr was responsible for the theoretical explanation, Svedberg cited articles that Bohr had published after the appearance of Meitner and Frisch's paper, and which built on their work.

The war created unusual circumstances for the evaluation of candidates. The prizes were deferred in 1940, cancelled in 1941 and 1942, and reserved in 1943. Finally, in 1944, the reserved 1943 prizes

handed the matter over to the chemistry committee. In 1943 Manne Siegbahn, chairman of the physics committee, attempted to reopen the question and nominated Hahn alone for the physics prize. Nuclear fission, he wrote, "lies on the boundaries between physics and chemistry". But the physics committee again decided to refer the evaluation to the chemistry committee.

The chemistry committee remained in charge of the matter until 1945. There were no external nominations, but Hahn's candidacy was kept active through a

de Hevesy had already been recommended for the 1940 prize in chemistry, it placed Hahn in line for the next award. Dissension arose within the committee in 1942. Wilhelm Palmaer proposed that the prize should be divided between Hahn and Meitner, whereas Westgren argued that it should be awarded to Hahn alone. Palmaer referred to arguments made in Svedberg's 1939 report and to Meitner's work in 1939 and later. However, when Palmaer died in June 1942, Westgren's opinion gained the upper hand.

That year, Westgren was charged with

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Beyond recognition: Lise Meitner and her collaborators Otto Frisch (left) and Fritz Strassmann (right).

were awarded in physics, chemistry and medicine. Invitations were sent out to nominators, although because of the war the invitations and replies did not always arrive. The committees also continued to evaluate and, to some extent, rank candidates. But wartime conditions made it difficult for committee members to consult informally with members of the international scientific community. So, more than in peacetime, they had to base their evaluations on articles in scientific journals, which often arrived after great delay.

Some international nominators urged that nuclear fission should be recognized by a Nobel prize in physics. The Nobel prize-winning physicist James Franck nominated Hahn and Meitner for a joint award every year from 1940 to 1943 and drew attention to the fact that Meitner had not "co-worked" the paper published by Hahn and Strassmann because she had been forced to leave Germany. She and Frisch were the first to see the "importance of the result" and to conclude that the fission products would "fly" with "tremendous energy".

In its 1941 general report, however, the physics committee noted that Hahn and Meitner had been nominated for a Nobel prize in chemistry and that their work appeared to "belong to chemistry", and

nomination made each year by the committee secretary Arne Westgren, professor of chemistry at the University of Stockholm, who became a committee member in 1943.

In 1941 the chemistry committee asked Svedberg to update his 1939 evaluation in view of the intense research activity in the field. The subsequent report listed more than 150 publications in leading journals dealing with various aspects of fission research. Svedberg also alluded to the possibility of a chain reaction and to the enormous energy it would release. He argued that "Hahn's discovery" was fundamentally important for nuclear chemistry.

By contrast, Svedberg noted that Meitner had not produced works of "great importance in the past two years" (while living in Sweden after having been forced to flee Germany) and claimed that the work she had published alone or together with Frisch in 1939 had not "significantly influenced developments in the field". Svedberg's bibliography listed Meitner and Frisch's *Nature* article giving the theoretical explanation for fission, but he again cited only Bohr's theoretical work in the text. The committee followed Svedberg's recommendation and agreed that a Nobel prize in chemistry should be awarded to Hahn alone. Because George

evaluating the respective merits of Hahn and Meitner. Westgren first attacked the argument that Meitner's and Hahn's joint researches had significantly contributed to the discovery of nuclear fission. Indeed, Hahn himself had not acknowledged Meitner's contributions, both theoretical and experimental, made in letters and in person, during the critical period just before the discovery of barium as a fission product.

Westgren then dismissed the claim that Meitner had contributed to the understanding of nuclear fission after its discovery. The Swedish chemist cited only Meitner's and Frisch's experimental work confirming the existence of fission products, and pointed out that such confirmation had been provided by many scientists. Once again, their theoretical explanation was not mentioned. Instead, Westgren reiterated Svedberg's opinion that Bohr had made the major contribution. He concluded that if Meitner had not been forced to break off her collaboration with Hahn in 1938 because of "unhappy circumstances", she would no doubt have participated in the investigations that led to the discovery of fission, and a joint award would have been "justified".

Westgren's firm stand and the committee's position on the issue of a divided

prize did not change during the rest of the war. After his election to the committee in 1943, Westgren moved to the key position of chairman and became permanent secretary of the academy. He proposed Hahn for foreign membership of the academy (an honour that, for other scientists, had preceded a Nobel prize) and may have arranged for him to give a talk at the academy when he visited Sweden in the autumn of 1943.

In 1944 the committee's general report recommended that Hahn should be awarded that year's prize for chemistry. At the same time, de Hevesy was put forward for the prize that had been reserved in 1943. The chemistry section and the academy went along with the committee's recommendation of de Hevesy, but they rejected the one for Hahn, and the 1944 prize was reserved until the following year. The committee's recommendation may have leaked out, for Hahn and others were now certain that the prize had been set aside for him.

Hahn's Nobel prize in chemistry was finally decided in 1945. There was still only one nomination in chemistry — Westgren's for Hahn. In physics there was now a smattering of nominations for the discovery of nuclear fission, including, most importantly, Meitner and Frisch by Oskar Klein, professor of theoretical physics at the University of Stockholm. Klein argued that the discovery of nuclear fission had a chemistry and a physics side and that Meitner and Frisch's theoretical explanation of the phenomenon made them co-discoverers together with Hahn. Furthermore, their article in *Nature* had been the foundation for all subsequent theoretical work, including that by Bohr and John A. Wheeler. At the end of February 1945 Klein was elected a member of the academy, putting him in close contact with decision-making about the prizes and, among other things, giving him access to the committees' general and special reports.

Klein's nomination forced the physics committee to grapple with the issue of rewarding Meitner and Frisch, while the question of a prize for Hahn remained with the chemistry committee. After preliminary discussion during the spring, Erik Hulthén, professor of physics at the University of Stockholm, spectroscopist and member of the Siegbahn school, was asked to evaluate Meitner and Frisch's contribution. Hulthén's three-and-a-half page memorandum, dated 9 June 1945, was based on the 1939 articles in *Nature* and *Naturwissenschaften*, the only information, as he pointed out, available to him. Hahn's and Strassmann's articles in *Naturwissenschaften* showed that they had grasped the process of fission "to the fullest extent". Even if Meitner and Frisch had made an important contribution to the understanding of the physical side of

fission, Hulthén concluded, so had many other researchers.

Hulthén's negative report led Klein to contact Bohr, the obvious person to appraise Meitner and Frisch's theoretical contribution. Klein felt that Meitner had not been treated fairly by Hahn. But Bohr, who had fled Denmark in October 1943, was still in the United States. Klein entrusted Hulthén's report to Bohr's wife, Margrethe, who was travelling to meet him, and asked Bohr to make a statement that he could present when the physics section met in October.

At the end of the war, Hahn and nine colleagues connected in varying degrees with the wartime nuclear fission project were rounded up by Allied forces, brought to England and interned at Farm Hall, a country manor near Cambridge. On 6 August they heard the news that the atomic bomb had been dropped on Hiroshima. Hahn was hit hard by the realization that nuclear fission had been used as a weapon of mass destruction. But it is perhaps most interesting to note that Hahn and his colleagues immediately began writing Meitner out of the discovery of nuclear fission as part of the apologetics they developed at Farm Hall⁴.

By contrast, the importance of Meitner and Frisch's theoretical contribution was clearly stated in the first reports on the Manhattan Project prepared by the British and US governments and published a month after Hiroshima. Bohr, who had returned to Copenhagen in late August, sent both reports to Klein. Bohr felt that simultaneous awards to Meitner and Frisch in physics and Hahn in chemistry, both men having been involved in atomic bomb projects but on opposite sides, could perhaps help the cause of international control of atomic energy. Klein contacted Hulthén to discuss the matter, but learned that the physics committee was going to recommend Wolfgang Pauli, a long-standing candidate for the prize. Klein now concentrated his efforts on the chemistry committee in the hope of stalling an award to Hahn in order to allow a re-examination of the discovery.

When the chemistry committee handed in its report to the academy in late September, it recommended unanimously that the decision should be deferred. Surprisingly, this was motivated not by a desire to re-evaluate the original discovery but "by the revelations which have been made lately in connection with the sensational news about the atomic bomb". The members of the committee seemed concerned that unpublished research carried out by Allied scientists in the Manhattan Project had escaped their notice. Putting off rewarding the discovery would enable them to gain more exhaustive knowledge about "contributions that might even be on a par with the original discovery".

As the November meeting of the full

academy drew closer, Klein had reasons to hope that his campaign to fend off an award to Hahn in chemistry would be successful. Despite Hulthén's negative evaluation, the physics committee had left open the matter of an award in physics for nuclear fission. When the full academy meets to decide the year's prize-winner, the discussion is normally a closely guarded secret and the vote is not recorded. But we know what happened in 1945 because of information contained in a letter Klein wrote to Bohr. There were two motions: one, that the 1944 prize in chemistry should be reserved; the other, that it should be awarded to Hahn. Westgren, and especially Svedberg, pleaded that the prize should be reserved because the committee's previous evaluations had been made without the information now available in the United States and France. Klein spoke along the same lines.

But the physiologist Göran Liljestrand, professor at the Karolinska Institute and an influential member of the academy, did not accept that argument and spoke strongly in favour of Hahn. Klein felt that other members, who did not speak at the meeting, were also disturbed by the abrupt reversal of Svedberg and Westgren, which they felt had been caused by political motives, especially the wish to be favourably viewed by the United States. When the vote was taken, slightly more than half the votes were cast in favour of the motion to award Hahn the prize.

Following this decision, efforts were made to rectify the injustice to Meitner by nominating her for a prize in either physics or chemistry, alone or together with Frisch. Fuller knowledge about why these and other nominations were not successful will have to await the year-by-year opening of the Nobel archives and, very likely, the private papers of the scientists involved. Any subsequent award to Meitner might have implied that the academy had made a mistake. In any case, it was much easier to close the books on the discovery of nuclear fission and move on to other scientists whose work also deserved a Nobel prize. □

Elisabeth Crawford is at CNRS Institut d'Histoire des Sciences, Université Louis Pasteur, F-67000 Strasbourg, France. Ruth Lewin Sime is in the Department of Chemistry, Sacramento City College, Sacramento, California 95822, USA. Mark Walker is in the Department of History, Union College, Schenectady, New York 12308-2365, USA.

1. Sime, R. *Lise Meitner: A Life in Physics* 326–329 (Univ. California Press, Berkeley, 1996).
2. Bohr Scientific Correspondence (Niels Bohr Archives, Copenhagen).
3. Crawford, E. *The Beginnings of the Nobel Institution: The Science Prizes, 1901–15* (Cambridge Univ. Press, Cambridge, 1984).
4. Walker, M. *Nazi Science: Myth, Truth, and the German Atomic Bomb* 207–241 (Plenum, New York, 1995).

Look Ma, no chromosomes!

Jeremy Hyams

The segregation of chromosomes at mitosis and meiosis during cell division depends on their interaction with a bipolar spindle. But, surprisingly, it turns out that the spindle can form and function even in the absence of chromosomes.

WHEN a eukaryotic cell divides, the segregation of two sets of chromosomes as the nucleus splits at mitosis with the formation of two daughter cells makes a fittingly dramatic end to one cell cycle and start of the next. Yet more than a hundred years on from Flemming's first detailed descriptions of these events, we are still struggling to understand the relationship between the chromosomes and the structure that choreographs their movements, the mitotic spindle. The problem is an attractive one in many respects, not least because images of chromosomes and spindles are inherently rather beautiful (see cover).

Although, for most of us, it is the behaviour of the chromosomes that defines the different stages of mitosis (prophase, prometaphase, metaphase, anaphase and telophase; see figure), there is a nagging uncertainty among mitosis professionals as to whether chromosomes are active participants in their own segregation, or merely passive onlookers. Mazia¹ articulated one extreme view very nicely when he likened chromosomes at mitosis to the corpse at a funeral — the object of much of the attention but playing no active role in the proceedings. This macabre analogy seems to be borne out by some rather remarkable experiments reported in this issue by Heald *et al.* (on page 420)² and by Zhang and Nicklas (on page 466)³, who show that the corpse can be substituted or even removed without disturbing the ceremonies.

Conventionally, the establishment of a functional bipolar spindle (see figure) is assumed to require two structures: spindle poles and chromosomal kinetochores. In animal cells, the poles are centrosomes which arise by duplication of the structure that organized the microtubule cytoskel-

eton during interphase, the period between divisions. Coincident with the condensation of the chromosomes inside the nucleus, the daughter centrosomes, which lie in the cytoplasm, move apart, and microtubules begin to polymerize and

the best possible chance of delivering a perfect set of chromosomes to each daughter cell is the hypothetical 'glue' holding the paired chromatids together dissolved and chromosome segregation (anaphase) initiated. The retreating chromosomes leave behind a

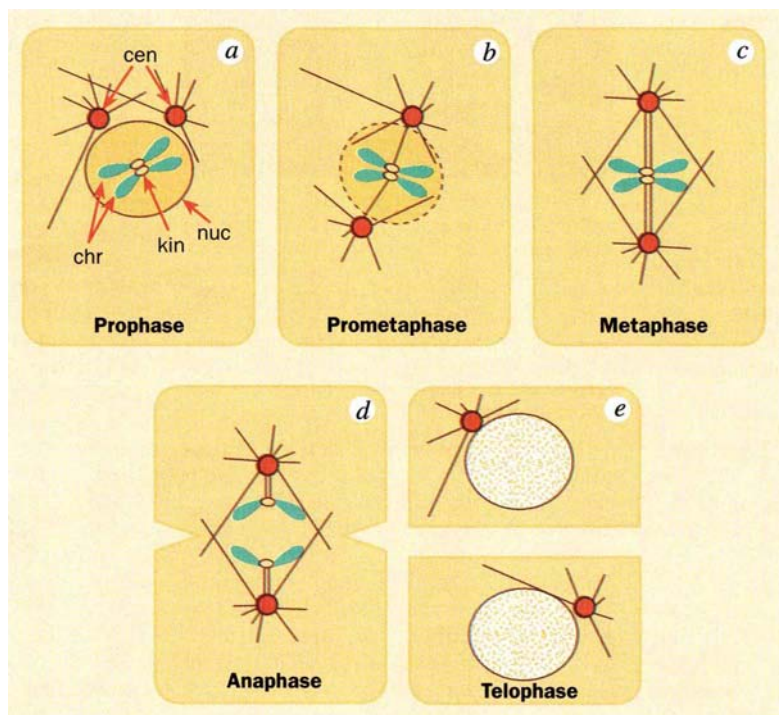
gap which is constricted by the advancing cleavage furrow that splits the cells in two.

Given the familiarity of this process to all biologists, a great many questions about mitosis remain to be answered. To what extent do chromosomes contribute to spindle formation and to their own movement at anaphase? Do they have a role in positioning the cleavage furrow? What holds sister chromatids together, how are they 'unglued', and what is the signal for this detachment? How do the checkpoints that sense a single detached chromosome or an imperfect spindle work?

In a series of experiments that took real balls, Heald *et al.*³ have attempted to address some of these issues based on the ability of egg cytoplasm from the frog *Xenopus* to assemble functional spindles when supplied with

other sources of chromatin. As a unique source of artificial chromosomes, they added magnetic beads that had been coated with plasmid DNA. Astonishingly, bipolar spindles were formed, with the spheres aligned across the equator in a manner strikingly reminiscent of a normal metaphase. Beads coated with another acidic polymer, polyglutamic acid, failed to form spindles.

Examination of early stages in the spindle-assembly process revealed that microtubules initially appeared as short, randomly oriented fragments in close proximity to the beads. Subsequently, these coalesced into bundles



Basic stages of mitosis. *a*, Prophase: centrosomes (cen) start to separate outside the nucleus (nuc); chromosomes condense within the nucleus. Each chromosome consists of paired chromatids (chr) attached by their kinetochores (kin). *b*, Prometaphase: nuclear envelope breaks down; chromosomes start to attach to the forming spindle. *c*, Metaphase: bipolar spindle forms; chromosomes align across the equator. *d*, Anaphase: kinetochores split and chromosomes move to poles; cytokinesis begins (indents). *e*, Telophase: chromosomes decondense, the nuclear envelope reforms and cytokinesis is complete.

depolymerize randomly and rapidly from them.

With the breakdown of the nuclear envelope, microtubules and chromosomes are free to interact. In a strictly hit-or-miss process, a subset of microtubules from each pole is captured and stabilized by the complex kinetochore structures that have by now formed at the chromosomal centromeres. The remaining microtubules intermingle to form a zone of overlap. Checkpoints monitor both the fidelity of spindle assembly⁴ and whether the chromosomes are all present and correct⁵. Only when the system is judged to have

that eventually reorganized themselves into an apparently perfect bipolar structure, even though neither centrosomes nor kinetochores were present. After monitoring the behaviour of labelled microtubule 'seeds' of known polarity in their assays and the effect of inhibitors of microtubule motor function, Heald *et al.* concluded that the role of chromosomes in spindle assembly is to provide an environment in which microtubule assembly is favoured. The establishment of a bipolar structure thereafter is a function of the intrinsic polarity of the individual microtubules and their translocation by the motor protein dynein. Strikingly, bipolar spindles failed to form in egg extracts depleted of dynein by pre-incubation with an anti-dynein antibody.

In a second blow for chromosome lovers, Zhang and Nicklas³ laid in wait for grasshopper spermatocytes to complete spindle assembly and then used a microneedle to remove all of the chromosomes. Amazingly, not only was 'anaphase' initiated on schedule in these chromosome-free spindles, but the spindle microtubules behaved in a way that was difficult to distinguish from control cells in which the chromosomes were still present. In a final bizarre twist, the chromosome-less cells also executed a faultless cytokinesis that divided the cell into two anucleate daughters.

These intuitively simple but technically brilliant experiments challenge a number of cherished ideas about mitosis. The concept of the kinetochore chomping its way along microtubules to the poles⁶, the argument that anaphase is triggered by the separation of sister chromatids⁷, and the possible role of chromosomes in the positioning of the cleavage plane⁸, all now have to be re-evaluated.

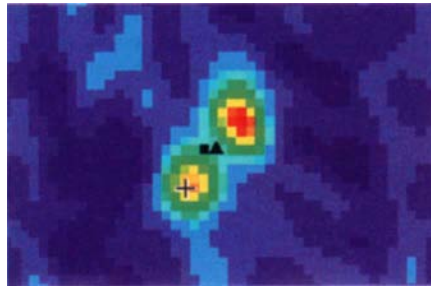
But don't be too despondent about all this. Every model ever proposed to explain spindle formation and chromosome segregation has probably been wrong, at least in detail. Even given the undoubted ingenuity of cell biologists, it might be a while before we can finally close the book on a process that we all know so well but understand so little. □

Jeremy Hyams is at the Institut de Pharmacologie et de Biologie Structurale, Université Paul Sabatier, 205 route de Narbonne, 31077 Toulouse, France, on leave from the Department of Biology, University College London, London WC1E 6BT, UK.

Obscured objects of desire

Richard Barvainis

QUASARS are rare. Once, before the Universe was half its present age, they were a thousand times more common. Looking back even further, however, they seem suddenly to become rare again. One implication of two studies reported in this issue^{1,2}, by Ohta *et al.* on page 426 and Omont *et al.* on page 428, is that those earliest quasars might just be hiding.



Carbon monoxide emission from around the redshift-4.7 quasar BR1202-1275, obtained using the IRAM Plateau de Bure Interferometer². The quasar is marked by a cross, and the square and triangle mark sources of faint optical continuum and Lyman- α emission associated with it. Its companion (or perhaps a gravitationally lensed image) lies north-west of the quasar, about 4 arcsec away (corresponding to 12–30 kiloparsecs).

The papers present detections of dust continuum emission and carbon monoxide line emission from one of the most distant quasars known, and also, quite unexpectedly, from an optically invisible companion object. The companion could be an image of the quasar produced by gravitational lensing, or, perhaps more interestingly, it could be an independent galaxy. In superseding previous distance records for molecular line emission^{3,4}, these findings show that elements heavier than hydrogen and helium were produced in great abundance through star formation in the very early history of the Universe. They also suggest a new means for exploring the Universe at distances even greater, and times even earlier, than currently afforded by classical quasars.

The primary subject of the new studies is a quasar called BR1202-0725, which lies at a redshift of 4.69. Redshift signifies both distance and look-back time, and the emission we see now left BR1202-0725 when the Universe was only about 7% of its current age (assuming a just-closed Universe), or roughly a billion years after the Big Bang. The large masses of dust and carbon monoxide gas in BR1202-0725 show that primordial hydrogen and helium gas had already been enriched with heavier elements on galaxy-wide scales at this early time. This enrichment occurs entirely by nuclear burning in the centres of stars, so there must already have been at least

one or two stellar generations in the galaxy surrounding BR1202-0725.

In their search for the earliest and most distant objects in the Universe, astronomers have been stalled for some time at a redshift (z) of just under five, with only very recent indications of galaxies at higher redshifts⁵. The current record-holder for definitively measured redshifts is a quasar at $z=4.89$. There is evidence⁶ that the classical quasar population dwindles sharply beyond a redshift of three, and intensive optical searches for quasars have not been successful in breaking the $z=5$ barrier. We are compelled to ask: what kinds of objects, if not optically bright quasars, might have existed before that? The large quantities of gas and dust in BR1202-0725, and in its companion (if it is real), may be a clue. Is it possible that the precursors to quasars and galaxies as we now define them were optically obscured by the products of early starbursts?

A flurry of star formation in a primitive galaxy would produce gas and dust enriched in heavy elements, spewed forth by millions of supernovae and stars in their giant phase, which could have formed a deep haze that both obscured the optical light from the stars and hid any quasar shining from within the galaxy's nucleus⁷. Most of the stellar and quasar output would be re-radiated in the far-infrared region (50–200 μm). Owing to the large redshifts, this emission would then be detectable from Earth at wavelengths as long as 1 mm (1,000 μm).

A similar situation has been seen in the case of young protostars in our own Galaxy. Searches in the optical and near-infrared regions were carried out for years without success, and as M. Barsony⁸ noted recently, "Like the Holy Grail, a [near]-infrared protostar was never found, because, as we now know, such objects radiate most of their luminosity at far-infrared and submillimetre wavelengths." Assuming that the companion object to BR1202-0725 — let's call it BR1202C — is an independent galaxy, it appears to have this same dominant far-infrared emission.

BR1202C looks similar to the much less distant 'ultraluminous infrared galaxies', which also emit mostly in the far infrared. Many of these infrared galaxies may be largely powered by hidden quasars. The discovery of massive concentrations of dust and gas at $z=4.7$, in BR1202-0725 and in its possible companion, makes more plausible the idea that the earliest galaxies and quasars were primarily far-infrared emitters much like local infrared galaxies. And Cosmic Background Explorer observations may support this idea: there is evidence for diffuse submillimetre

S. Guilloteau

1. Mazia, D. in *The Cell* Vol. III (eds Brachet, J. & Mirsky, A. E.) 77–412 (Academic, New York & London, 1961).
2. Heald, R. *et al.* *Nature* **382**, 420–425 (1996).
3. Zhang, D. & Nicklas, R. B. *Nature* **382**, 466–468 (1996).
4. Sluder, G. J. *Cell Biol.* **80**, 674–691 (1979).
5. Rieder, C. L. *et al.* *J. Cell Biol.* **127**, 1301–1310 (1994).
6. Inoue, S. & Salmon, E. D. *Mol. Biol. Cell* **6**, 1619–1640 (1995).
7. Funabiki, H. *et al.* *Nature* **381**, 438–441 (1996).
8. Earnshaw, W. C. *et al.* *Cold Spring Harb. Symp. Quant. Biol.* **56**, 675–685 (1991).

background radiation, consistent with a large population of dust-obscured galaxies at high redshift⁹.

But how will we find these objects? And how, without the traditional tool of optical emission lines, will we determine their precise redshifts? The answers may lie with two new instruments, one already built and about to begin observations, and the other currently under construction and due for completion in less than two years.

The first instrument, called SCUBA (for Submillimeter Common-User Bolometer Array), will be used on the James Clerk Maxwell Telescope on Mauna Kea, Hawaii. It is a camera designed to work in the 350–850- μm range with unprecedented speed and sensitivity. Large-scale sky surveys may turn up optically obscured objects that look much like BR1202C, and, if so, redshifts can be very crudely estimated from the shape of their submillimetre continuum spectra. But it will be hard to tell definitively whether the redshifts are beyond the $z=5$ boundary.

That is where the second instrument comes in. The Green Bank Telescope is a 100-m dish being built in West Virginia by the US National Radio Astronomy Observatory. With its large collecting area, sensitive receivers and wide spectral bandwidth, just a few hours of observing should be needed to detect CO lines in objects like BR1202–0725 or its companion in the $z=5$ –10 range. It should be feasible to search for lines in the wavelength range 9–6 mm, or 35–50 GHz, and determine redshift that way—the CO rotational transitions are separated by 115 GHz in the rest frame, but this separation becomes compressed by a factor of $1/(1+z)$, so for $z=8$, for example, there is a CO line every 12 GHz.

Of course, it is impossible to say whether we will discover obscured versions of quasars with properties like BR1202C at higher redshifts, even with the most powerful new instruments. But the wait should not be too long. For now we are free to speculate that this may be what primitive quasars and galaxies looked like in the infancy of the Universe. □

Richard Barvainis is at the MIT Haystack Observatory, Route 40, Westford, Massachusetts 01886, USA.

Communication between oceans

Arnold L. Gordon

THE nature and the very existence of a coherent global ocean-circulation pattern has been in debate for the past 10 years¹. Particularly contentious is the relative importance of the 'warm route' and the 'cold route'—the possible paths of flow into the Atlantic Ocean needed to balance the export of North Atlantic Deep Water^{2–5} (see figure). On page 436 of this issue⁶, Macdonald and Wunsch present a coherent, global look at ocean circulation and heat flux that may answer that question. They suggest that instead of just one, there are two global-scale ocean-circulation cells, which behave nearly independently of each other.

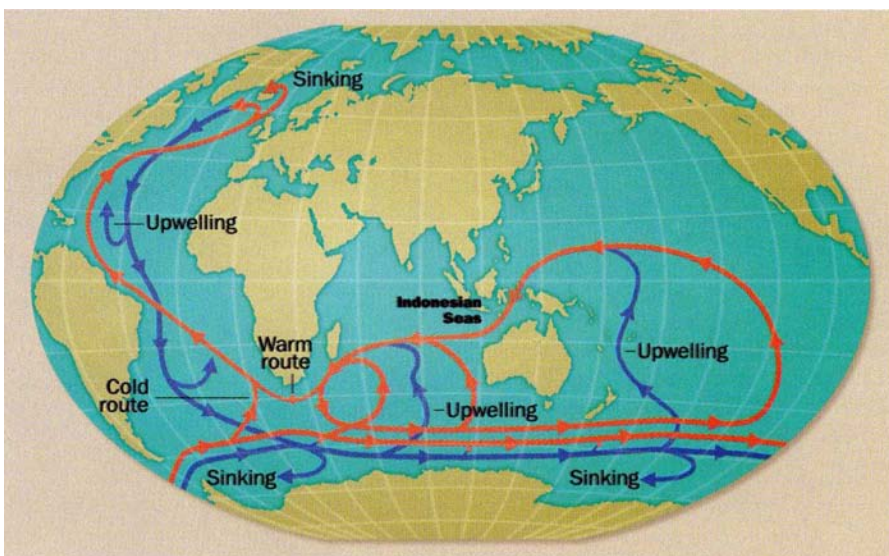
The global component of ocean circulation is usually thought to be driven by temperature and salinity differences (and called thermohaline circulation), while basin-scale flow (within the Atlantic or the Pacific, say) is considered to be wind-driven. But it is artificial to divide the circulation into these components, because nature satisfies all the forcing mechanisms with a single integrated circulation.

Ocean circulation transfers heat and fresh water between different climate regimes and between oceans, and it is coupled to convective overturning, which links the full ocean volume to the climate at decade-to-century timescales. For example, surface water sinks into the deep layers of the northern North Atlantic and draws warm surface water from the south, warming the European climate. Unless the sinking regions, principally the North

Atlantic, are compensated by upwelling within the confines of their local basin (which does not appear to be strong enough), inter-ocean circulation is implied. But what is its form, and how does it feed back into the convective processes and their associated climate phenomena?

Each of the oceans is exposed to different atmospheric forcing, and takes on a distinct stratification in temperature and chemistry. In the North Pacific there is more precipitation than evaporation, so the thermocline (the upper, warmer layer of any ocean) is low in salt, with limited, shallow convection; in contrast, the Indian and Atlantic are evaporative oceans, so their upper layers are more saline than those of the Pacific. In the northern North Atlantic, the dense, salty surface water causes deep convection at surface ocean temperatures well above the freezing point of sea water. Even the Southern Ocean, which is less constrained by geometry, has considerable longitudinal variations of near-surface salinity and associated bottom-water formation rates and properties.

The chemical and thermal differences between the major ocean basins would be much larger were it not for passages permitting inter-ocean exchange. These passages are constrictive links in the circulation's ability to equalize ocean properties between basins. Only by viewing the global nature of ocean circulation within the major basins and connecting passages can the ocean's role in the climate system be fully appreciated.



The early, simplified schematic of inter-ocean thermohaline circulation, driven by sinking of surface water in the North Atlantic^{1,3,9}. Blue lines are water at less than 3.5 °C, characterizing deep flow. Red lines are water at more than 3.5 °C flowing near the surface. The Pacific and Indian oceans are linked to the Atlantic overturning cell via a warm-water route south of Africa. But Macdonald and Wunsch's global ocean-circulation scheme indicates that, within the constraints of the steady-state assumption, this link is weak and intermittent, and independent of the Indonesian throughflow, effectively severing a single global circulation into two large-scale cells.

- Ohta, K. et al. *Nature* **382**, 426–428 (1996).
- Omori, A. et al. *Nature* **382**, 428–431 (1996).
- Brown, R. L. & Vanden Bout, P. *Astrophys. J.* **397**, L19–L22 (1992).
- Barvainis, R., Tacconi, L., Antonucci, R., Alloin, D. & Coleman, P. *Nature* **371**, 586–588 (1994).
- Lanzetta, K. M., Yahil, A. & Fernandez-Soto, A. *Nature* **381**, 759–763 (1996).
- Schmidt, M., Schneider, D. P. & Gunn, J. E. *Astron. J.* **110**, 68–77 (1995).
- Mazzei, P. & De Zotti, G. *Mon. Not. R. Astron. Soc.* **279**, 535–544 (1996).
- Barsony, M. in *Clouds, Cores, and Low Mass Stars* ASP Conf. Ser. Vol. 65 (eds Clemens, D. & Barvainis, R.) 197–206 (1994).
- Puget, J.-L. et al. *Astron. Astrophys.* **308**, L5–L8 (1996).

Macdonald and Wunsch estimate volume flux and heat flux around the world ocean using data from a set of 15 high-quality, bottom-reaching, trans-ocean sections, taken from 1,627 oceanographic stations. As the problem is underdetermined, a "certain amount of subjective oceanographic opinion" is applied. This ingredient is the insight that has been the hallmark of successful field oceanographers.

Within the limits of the data set, two independent global circulation cells emerge. The Atlantic/Southern-Ocean cell carries North Atlantic Deep Water into the Southern Ocean, where it is mixed by the active vertical fluxes and spreads into the Indian and Pacific oceans. The importance of deep-water upwelling activity within the Southern Ocean and its relationship to the sinking regions is becoming increasingly apparent¹.

The second cell is more confined to a horizontal plane, linking the Pacific and Indian oceans across the Indonesian Seas. Most of the exchange in this cell occurs in water warmer than 3.5 °C — roughly the upper 1.5 kilometres in the Pacific and Indian oceans. However, the model⁶ shows that around three million tonnes a second of deep, cold water slips into the North Pacific, where it wells up to pass into the Indian Ocean via the Indonesian Seas. As the flow into the North Pacific is partly composed of deep circum-Antarctic water, the deep-water limbs of the two cells are intertwined, albeit weakly, in the Pacific.

Thermocline water drawn from the Indian Ocean passes into the Atlantic after rounding the southern rim of Africa³, mainly in large eddies and filaments. Much of this exchange may be limited to the Agulhas recirculation cell within the southwest Indian Ocean⁶. However, there may be a contribution of around three million tonnes a second from the Pacific/Indian circulation cell^{3,5} linking the two global cells in the warm, upper limb. Macdonald and Wunsch's results for this region are less clear, owing to the highly time-dependent local conditions, but some intermixing of waters from their two giant cells is implied, within the southwest Indian Ocean and along the boundary between the two warm westward flows (double line in the figure). Furthermore, new work⁸ suggests a direct link between Agulhas eddies and the intensity of global thermohaline circulation.

The thermohaline-circulation schematics of a decade ago have been challenged as being too simple, and so a series of authors^{5,6} have added more internal circulation loops that capture the true turbulent nature of the ocean. While their depictions are probably correct, they tend to obscure those weak but perhaps climatically potent elements of the global ocean circulation that initially prompted the use of simple schematics. Might the weak, time-varying links be important in maintaining two semi-independent circulation cells? Or is each self-contained in terms

of heat and freshwater balance?

The vigorous intra-ocean gyres are not steady-state features, particularly at their boundaries, and their variations may swamp the inter-ocean fluxes and associated global circulation cells (which themselves are probably not constant). Oceanographers now need a global data-collection net, capable of detecting the pulses of change. □

Arnold L. Gordon is at the Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York 10964, USA.

EVOLUTION OF FLIGHT

Early bird in slow motion

Kevin Padian

THE most recent evidence to shed light on the evolution of flight in birds is described by Sanz *et al.*¹ on page 442 of this issue — their remarkable discovery of the first example of a preserved alula, or bastard wing, in a 125-million-year-old bird from the Lower Cretaceous of Spain (see Fig. 1). The alula is functionally equivalent to the leading-edge flaps of an aeroplane, which allow flight at low speeds without stalling. The new find confirms that the wing mechanism that allows modern birds to fly and manoeuvre at low speeds must have evolved early in their history.

Consensus developed almost two decades ago that birds evolved from small carnivorous dinosaurs of the Jurassic, theropods similar to *Velociraptor* which ran along the ground and seized their prey with outstretched arms² (see Fig. 2). It was a short jump from this stage to that of the famous *Archaeopteryx*, which could definitely flap, but probably not for long distances, and was not much of a glider. It turns out that the grasping, predatory

stroke of the small theropod dinosaurs was very similar to the down-and-forward forelimb motion that propels birds in low-speed flight³. These features, plus the ecological context of the theropods from which birds evolved, provide no evidence that bird ancestors were arboreal, and give no indication that all-out gliding was a way-station on the road to flapping flight. But the newest discoveries in avian evolution have helped to fill in the next stops on the way.

Fifteen years ago, Cyril Walker of the Natural History Museum in London gave a name to a collection of Cretaceous birds from Argentina which had strange shoulder girdles, warped foot bones, and a humerus with a large hole in it⁴ — little did he realize that these Enantiornithes (or "opposite birds", as he called them) were soon to become the best known and most diverse group of birds in the Cretaceous world⁵. Enantiornithes in a variety of sizes have so far hailed from six continents and a diversity of habitats, filling in

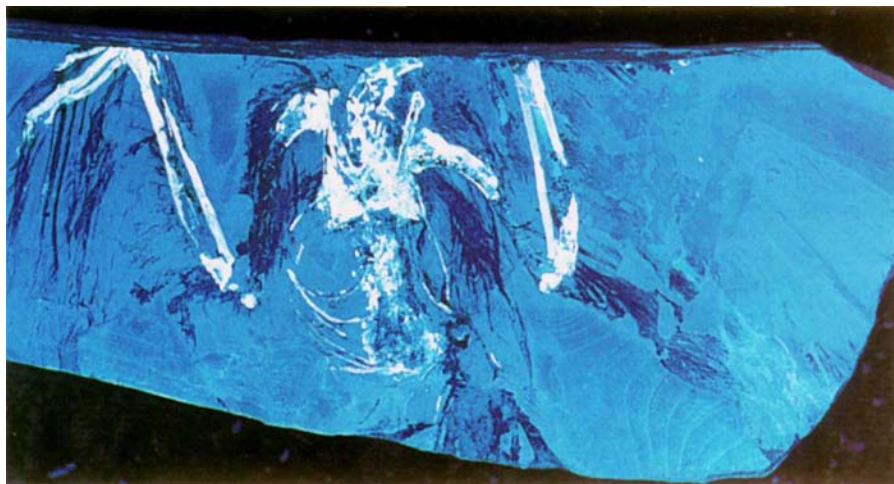


FIG. 1 Ultraviolet fluorescence image (before preparation) of the new Lower Cretaceous bird *Eoalulavis hoyasi*. The bones are highlighted in white and the feathers are dark blue. Wing feathers are seen attached to the 'hand', including the bastard wing, or alula, which is the uppermost digit in the photograph. (Photograph courtesy of G. F. Kurtz.)

- Gordon, A. L. *J. Geophys. Res.* **C 91**, 5037–5046 (1986).
- Rintoul, S. J. *Geophys. Res.* **C 96**, 2675–2692 (1991).
- Gordon, A., Weiss, R. F., Smethie, W. M. Jr & Warner, M. J. *J. Geophys. Res.* **C 97**, 7223–7240 (1992).
- Saunders, P. M. & King, B. A. *J. Phys. Oceanogr.* **25**, 1942–1958 (1995).
- Schmitz, W. J. *Rev. Geophys.* **33**, 151–173 (1995).
- Macdonald, A. M. & Wunsch, C. *Nature* **382**, 436–439 (1996).
- Cai, W. & Baines, P. J. *Geophys. Res.* **C 101**, 14073–14093 (1996).
- Schmitz, W. J. *J. Geophys. Res.* **C 101**, 16259–16271 (1996).
- Broecker, W. S. *Oceanography* **4**, 79–89 (1991).

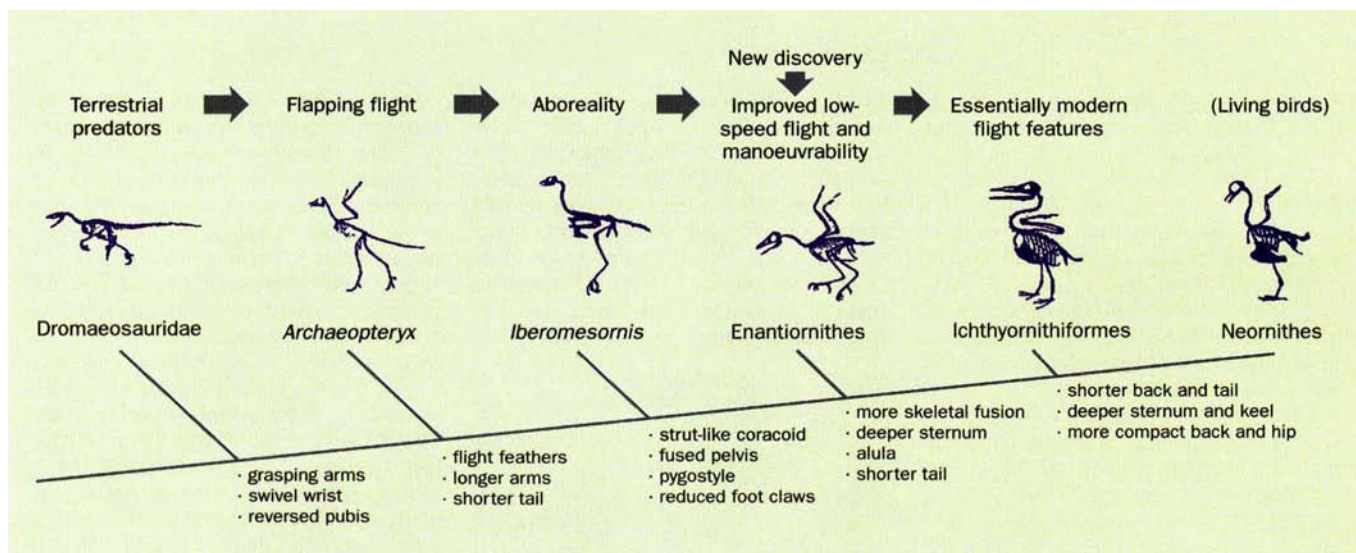


FIG. 2 Evolution of flight features in birds. For synapomorphies (shared, derived characters) uniting these taxa, see Fig. 1 of ref. 9.

a gap of some 50 million years (Myr) in the bird fossil record. Before their discovery, there was virtually only a handful of remains between *Archaeopteryx* and two early Late Cretaceous birds that had been known since 1880—the flightless, loon-like diver *Hesperornis*, and the smaller, tern-like *Ichthyornis*⁶, neither of which provided much information about the evolution of flight.

The recently discovered seventh specimen of *Archaeopteryx* preserves a partial, rectangular sternum, long suspected but never previously documented⁷. This attests to its strong flight muscles, but its capacity for long flights is questionable. Its shoulder joint may not have been angled as in later birds, it does not seem to have had the reverse-pulley mechanism of the supracoracoideus muscle, which raises the wing in flight, and its hand and wrist bones were not yet fused. So, even if it had the equipment and could perform the motions of flight, it was not locked into this function to the exclusion of others. And it still retained the long tail (somewhat reduced and heavily plumed) of its dinosaurian ancestors, which probably helped to stabilize it in the air. However, its claws are as similar to those of ground birds as they are to those of climbing or perching birds, and it has no obvious arboreal adaptations⁸.

If *Archaeopteryx* was a good runner with moderate flapping ability that could take off from level ground as well as from low heights, what happened next in the evolution of flight? Since 1981, most discoveries of Cretaceous birds have been of Enantiornithes, or of even more basal birds⁹, and these have addressed many of the deficiencies in the early bird record. *Noguerornis*, from the lowest Cretaceous of Spain, already has longer forearms and interlocking hand bones to facilitate flight. *Iberomesornis* (which, like the new bird

described by Sanz *et al.*¹, comes from the remarkable Las Hoyas deposit in Spain, and is only slightly younger than *Noguerornis*) has a strut-like coracoid clearly braced to the sternum, a tail reduced to a series of free vertebrae and a pygostyle (the fused bony component of the 'Parson's nose'), and a foot with recurved claws and a fully descended hallux (first toe), which clearly indicate its perching abilities. This is important, because before the new fossil bird discoveries of the past fifteen years, there was very little convincing evidence that birds had arboreal habits much before the Early Tertiary (60 Myr ago); now they can be established at twice that age.

Enantiornithes such as the new find described by Sanz and co-workers¹ share with later birds some more derived flight features. There is increased fusion in the hand and foot bones and in the hip, the free tail becomes shorter, and the sternum is deeper, with a more pronounced keel. The new Las Hoyas bird clearly shows for the first time an alula, a wing slot that breaks up boundary-layer turbulence so that the wing can function at the high angle of attack necessary for low-speed flight (and hence allow increased manoeuvrability). *Archaeopteryx* apparently lacked this, though it was evidently not a high-speed flier, so it must have been less manoeuvrable or used some alternative strategy, such as running landings. It seems certain that landing on a tree branch would have been much more difficult than it was for more derived birds.

It is likely that the alula and other flight-related advances contributed to the success of the Enantiornithes, but there is no record of them after the penultimate stage of the Cretaceous. By that time, all but the finishing touches on flight evolution seem to have been in place. Birds closer to our living forms, such as the Late Cretaceous *Ichthyornis*⁶, had a shorter

back and tail, more compact hip, and a deeper sternal keel. The flight-related characters of birds, particularly those of the forelimb, were modernized before the hindlimb and other features (including loss of teeth)⁵. This correlates with observations¹⁰ that in avian hindlimb evolution, knee flexion replaced hip extension as the primary kinematic mode. This in turn appears to be related to the reduction of the tail in theropod evolution, and the shift from its role as an anchor for the propulsive muscles of the hindlimb to the flight-related functions that it serves in birds.

So it seems that most avian flight advances were in place by the end of the Early Cretaceous, with fine-tuning through the remainder of the period. Ironically, like most technological pioneers, the bird groups that invented these features were victims of the success of their progeny, and were replaced by them before the end of the age of dinosaurs⁹. □

Kevin Padian is in the Museum of Paleontology, University of California, Berkeley, California 94720-3140, USA.

1. Sanz, J. L. *et al.* *Nature* **382**, 442–445 (1996).
2. Ostrom, J. H. *Biol. J. Linn. Soc.* **8**, 91–182 (1976).
3. Gauthier, J. A. & Padian, K. in *The Beginnings of Birds* (eds Hecht, M. K., Ostrom, J. H., Viohl, G. & Wellnhofer, P.) 185–197 (JuraMuseums, Eichstatt, 1985).
4. Walker, C. A. *Nature* **292**, 51–53 (1981).
5. Chiappe, L. M. *Courier Forschungsinstitut Senckenberg* **181**, 55–63 (1995).
6. Marsh, O. C. *United States Geological Exploration of the 40th Parallel 1–201* (Government Printing Office, Washington, 1880).
7. Wellnhofer, P. *Archaeopteryx* **11**, 1–48 (1993).
8. Peters, D. S. & Görgner, E. *Sci. Ser. Nat. Hist. Mus. Los Angeles Co.* **36**, 29–37 (1992).
9. Chiappe, L. M. *Nature* **378**, 349–355 (1995).
10. Gatesy, S. M. in *Functional Morphology in Vertebrate Paleontology* (ed. Thomason, J.) 219–234 (Cambridge Univ. Press, 1995).

George D. Snell (1903–96)

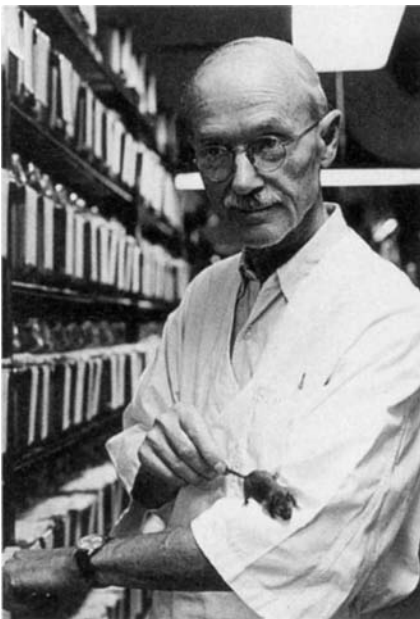
GEORGE Snell is dead. But — to paraphrase what was said about another man at another time — if I choose, I do not have to believe it. So profound has been his influence on an entire scientific discipline, that for as long as immunologists use congenic strains in their experiments, as long as the major histocompatibility complex continues to absorb us, indeed, as long as immunogenetics continues to exist, George Snell will remain with us in spirit.

Snell once remarked that had a course in astronomy been offered at the Harvard Medical School, where he enrolled in 1926, he might have become an astronomer. Instead, he was caught up in the enthusiasm of William E. Castle, a pioneer in mammalian genetics. At the Bussey Institute, an old mansion donated to Harvard by a wealthy family, Castle lived with his students and scores of rats, mice, guinea-pigs and rabbits. Snell was assigned the task of measuring linkage between the *dilute* and *short ear* genes in the house mouse. This assignment was the second decisive moment in Snell's career because — except for a brief period in which he studied the genetics of social behaviour in the parasitic wasp *Habrobracon* — he remained faithful to the mouse until he retired from experimental work.

Upon earning his PhD in 1930, Snell was granted a postdoctoral position with Hermann J. Muller at the University of Texas at Austin. A few years earlier, Muller had discovered that X-rays induced mutations in *Drosophila* and Snell proposed to test whether they did so in the mouse as well. Snell's two years at Austin proved, on the one hand, to be disappointing: he saw little of Muller, who spent part of the time on sabbatical leave in the Soviet Union, and he was unable to induce mutations in mice. On the other hand, he was exposed to *Drosophila* genetics and he discovered that X-rays induced translocations. Snell's work established that, at least in mammals, X-rays caused chromosomal aberrations rather than point mutations, it generated the methodological and conceptual basis for mammalian cytogenetic studies, and it produced several translocation strains (some still extant) that later proved useful in mapping studies.

Snell left Austin, was unemployed briefly, and then taught for a time before joining Clarence C. Little at the Jackson Laboratory, Bar Harbor, Maine, in 1935. Little, also a former student of Castle's, had founded the laboratory only six years earlier to study the genet-

ics of cancer, using mice as experimental animals. One of Snell's first tasks was to edit a book summarizing the available knowledge about the laboratory mouse. Because little was known about mouse embryology, and there was no one who could write this part of the book, Snell characteristically decided to research the area himself. The result was a chapter that



some 40 years later would become the foundation of the burgeoning discipline of mammalian developmental biology.

The editing of the *Biology of the Laboratory Mouse* had, however, another, more far-reaching effect on Snell's scientific career. Little contributed to the book a chapter entitled "The genetics of tumor transplantation", in which he summarized studies begun at the turn of the century and in which he had become involved while at the Bussey Institute. The studies stemmed from the observation that a spontaneously arisen tumour in a mouse of one inbred strain could be transplanted successfully to same-strain individuals but not to different-strain mice. Breeding experiments revealed the transplanted tumour growth to be controlled by multiple, but still unidentified, genes. Upon reading this chapter, Snell became fascinated by the mysterious tumour-resistance genes. He realized, however, that if he was to understand the nature of the histocompatibility genes, as he had begun to call them, they would have to be separated from one another, and he began to consider how this could be done.

His earlier contact with the *Drosophila* geneticists may have given him the advantage here. In 1948, he published a paper in which he described a method for developing congenic mouse strains, each of which would differ from an inbred strain at a single histocompatibility locus. He developed the mathematical theory of the underlying process, estimated how many years it would require and then began the work. Although a fire at the laboratory a couple of years later destroyed his initial efforts, he nevertheless began anew and ultimately produced the basic stock of mouse congenic strains differing at individual histocompatibility loci. Testing of the strains soon convinced him that the loci fell into two different categories — major and minor — depending on their effect on transplant survival.

Collaboration with Peter A. Gorer, who was visiting Jackson Laboratory on leave from Guy's Hospital in London, resulted in establishing that the major locus was identical to a locus encoding antigen I_I, identified earlier by Gorer. Snell and Gorer named the locus histocompatibility 2, or H-2. When further analysis revealed the locus to be complex genetically, the concept of the major histocompatibility complex, or MHC, was born. Although Snell followed several other leads stemming from his work with tumours (for example, he discovered independently the phenomenon of immunological enhancement), he and his co-workers focused mainly on further characterization of the H-2 immunogenetic complex. Much of the current knowledge concerning it derives from this work.

The concept of congenic strains and the discovery of the MHC has had a profound effect on the development of the entire field of immunology over the past 25 years. For these contributions Snell was awarded, together with Jean Dausset and Baruj Benacerraf, the Nobel Prize for Medicine in 1980.

George D. Snell died on 6 June. To future generations, he has left his scientific legacy; to those of my generation who knew him personally, he has bequeathed warm memories of a man modest to a fault, a man of the highest personal integrity, and one whose friendship brightened our lives. Jan Klein

Jan Klein is in the Abteilung Immunogenetik, Max-Planck-Institut für Biologie, Corrensstr. 42, D-72076 Tübingen, Germany.

Rust marks second-hand crust

William M. White

MAGMAS that erupt onto oceanic islands differ in composition from those produced at mid-ocean ridges. The differences are subtle, and largely restricted to trace elements and radiogenic-element isotope ratios, but they are important because they reflect differences in the composition of the mantle that melts to produce these magmas. Such traces provide persuasive evidence¹ for the idea that most oceanic islands are produced by mantle plumes² — columns of hot rock rising from deep inside the Earth. In contrast, magmas erupted at mid-ocean ridges are thought to be produced by melting of shallow upper mantle, welling up only because the crust above is moving apart. But are there also differences in the abundances of the major oxides? That would tell us a lot about the physics and origin of mantle plumes, but it is a notoriously difficult question to answer. On page 415 of this issue³, Hauri reports perhaps the best evidence yet that the major-element chemistry of the Hawaiian mantle plume does indeed differ from that of the upper-mantle source of mid-ocean-ridge basalts (MORB).

Demonstrating differences in major-element composition has been difficult for several reasons. A broad range of mantle compositions will produce similar melt compositions (melting is said to be 'eutectic-like'). Also, the temperature and pressure of melting affect composition. Finally, most magmas begin to crystallize as they rise, and segregation of these crystals changes the composition of the magma. The chemical effects of this crystallization are enough to mask subtle differences in primary melt composition.

Radiogenic isotope ratios are not affected by any of the above, and trace-element abundances are not changed enough to overwhelm the differences due to mantle composition. So rather than attempting to separate all the effects on major-element composition, Hauri approached the problem by looking for correlations between radiogenic isotope ratios and major-element abundances from a number of Hawaiian volcanoes. Those major-element abundances that correlate with isotope ratios should reflect differences in magma source composition, because variations in radiogenic isotope ratios in magmas can be due only to isotopic variations in magmatic sources.

Hauri acknowledges that reactions between rising magma and either the lithosphere or the crust under Hawaii might also produce correlations between isotope ratios and major-element concentrations. He argues, however, that his data

do not show the features characteristic of crustal assimilation, and that the observed correlations are opposite to those expected for reaction between magma and lithosphere. He thus concludes that "the most straightforward explanation for the correlations" is correlated differences in isotope ratios and in relative oxide abundances between the upper mantle and the Hawaiian plume, as well as within the plume. Hauri's conclusion that the plume is richer in iron oxide than the upper-mantle source of MORB is consistent with several earlier speculations. For example, Langmuir and Hanson⁴ found that FeO concentrations in Hawaiian basalts are higher than in MORB. They concluded that either the Hawaiian plume is richer in FeO than the upper mantle, or the depth at which melting occurs is much greater beneath Hawaii than beneath mid-ocean ridges.

All this is important for at least two reasons. First, the major-element composition, particularly iron concentration, affects the physical properties of rock, such as its density and seismic velocity. Up to now, attempts to model the dynamics of plumes and physically image them using gravity and seismic velocity have been made without firm knowledge of their intrinsic density — Hauri's results should therefore be of great interest to seismologists and geodynamicists. Second, major-element composition provides a key test of theories of the origin of mantle plumes.

Two models have dominated the thinking of geochemists over the past decade or so. In the first, proposed by Hofmann and White⁵, the distinctive isotopic and trace-element character of plumes reflects the presence of oceanic crust in them. Subducted oceanic crust sinks because it is cold and iron-rich, and therefore dense. In the deep mantle, perhaps at the core-mantle boundary, this material mixes with surrounding rock and is heated until it becomes light enough to rise as a mantle plume.

In the second model, proposed by McKenzie and O'Nions⁶, lithosphere beneath continents becomes enriched in some trace elements by melts that rise from below and freeze there. Under some circumstances, such as the sort of continent-continent collision that is happening between India and Asia, the cold lithosphere may delaminate and sink to the

deep mantle. Eventually it is reheated and rises as a mantle plume.

The Hofmann and White model predicts that plumes differ from the upper mantle in major-element composition, but because the addition of a small amount of melt to the lithosphere will have little effect on major-element abundances, the McKenzie and O'Nions model does not. Hauri argues that variations in major-element composition, and particularly their correlation with osmium isotope ratios, are precisely what would be

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

A piece of eclogite, showing brown grains of garnet and white quartz crystals. A similar rock, derived from subducted ocean crust, may exist in mantle plumes, mixing with the mantle to form the magmas that eventually erupt on island chains such as Hawaii.

expected from variable amounts of recycled oceanic crust within the Hawaiian plume. Thus, the case for oceanic crust recycling, which has been growing in recent years, has had another boost.

Hauri suggests that the recycled oceanic crust is present as lenses of quartz eclogite (a mixture of garnet and pyroxene) embedded within peridotite (an olivine-pyroxene-garnet rock that composes most of the upper mantle). The eclogite melts to produce a dacitic (SiO₂-rich) magma, which later mixes with basaltic magma produced by peridotite melting. The standard picture of the production of ocean-island magmas involves peridotite melting only, so this more complex picture would complicate the task of understanding

1. Hauri, E. H. *Nature* **382**, 415–419 (1996).
2. Schilling, J.-G. *Nature* **242**, 565–571 (1973).
3. Morgan, W. J. *Nature* **230**, 42–43 (1971).
4. Langmuir, C. H. & Hanson, G. N. *Phil. Trans. R. Soc. Lond. A* **297**, 383–407 (1980).
5. Hofmann, A. W. & White, W. M. *Earth Planet. Sci. Lett.* **57**, 421–436 (1982).
6. McKenzie, D. P. & O'Nions, R. K. *Nature* **301**, 229–231 (1983).
7. White, W. M. *Geology* **13**, 115–118 (1985).

magma genesis; fortunately, it is different enough to be easily tested.

Hauri's results may prove to be a turning point, inspiring new observational and theoretical studies, although laboratory melting experiments and thermodynamic modelling are needed to test it. Geophysicists can incorporate these results into revised models of dynamics and seismic velocity. Meanwhile, geochemists must examine other oceanic-island volcanoes for similar relationships.

We know from isotopic studies that there are several compositional classes of plume⁷, so it will be interesting to see whether the same relationships between isotope ratios and major elements exist elsewhere. We may find that ocean-crust recycling is not the only way plumes are made. □

William M. White is in the Department of Geological Sciences, Cornell University, Ithaca, New York 14853, USA.

EARLY LIFE

Some liked it hot

E. G. Nisbet and C. M. R. Fowler

THREE of the more intractable questions in palaeontology are the origin of sexual reproduction, the origin of photosynthesis, and the origin of life itself. Recent work by D. W. Grogan, reported in the *Journal of Bacteriology*¹, on genetic exchange in Archaea at high temperatures should send geologists back to their models of the earliest environments to see if they can accommodate the earliest living communities and to search for the circumstances in which modern biological processes began.

There is strong evidence that life on Earth is divided into three primary domains, Archaea, Bacteria and Eucarya² (see figure). The root of our own Eucarya line appears to lie closer to the Archaea than the Bacteria^{3,4}. A striking feature of the phylogenetic tree is that all the most deeply rooted branchings are between modern hyperthermophiles⁵, organisms that live at temperatures of 80–110 °C or more, and which are typically unable to grow below about 60 °C. By implication, the last common ancestor of life, or, perhaps the last common ancestral population, was probably (though not certainly)⁶ hyperthermophile. It is likely also that the ancestral line that led to the Archaea and Eucarya was also hyperthermophile. Grogan's work¹ shows that even at extremely high temperatures, the exchange of genetic information can occur in a member of the Archaea, *Sulfolobus acidocaldarius*, implying that populations of this organism have a partially sexual character. Chromosomal mutations arising in one

cell lineage can be transferred to other lineages. Perhaps the ancestral population that gave rise to the Archaea and Eucarya may have had this ability too.

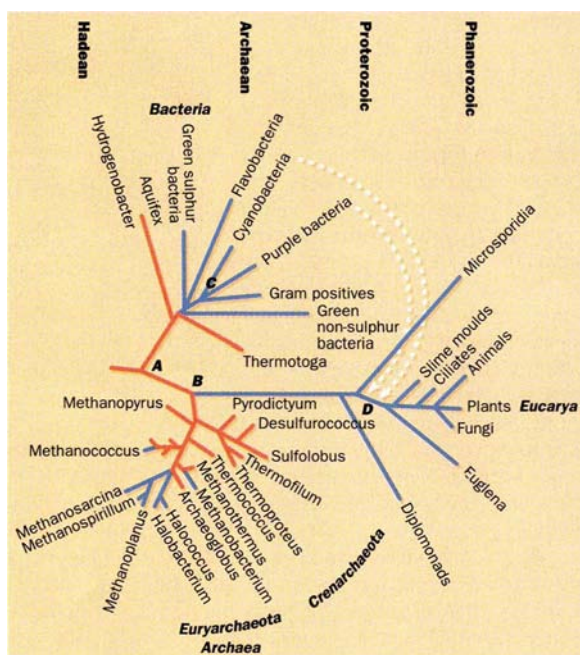
What does the geological evidence say? The Earth formed about 4.56×10^9 years (Gyr) ago. It is very unlikely that any life

3.5-Gyr-old rocks, and probably explains carbon isotope ratios in 3.8-Gyr-old metasediments in Greenland⁸. Possible cyanobacterial stromatolites and microfossils may be present in the Pilbara (Western Australia) and the Barberton Mountain Land (South Africa), though their identification is controversial^{9–11}. By about 3 Gyr ago, both macrostructures and isotope ratios in Archaean carbonates clearly show that photosynthetic life was both widespread and abundant, for instance at Steep Rock, Ontario¹². Thus, geologists would date the branching point marked C in the figure as very probably before 3.5 Gyr ago, and perhaps before 3.8 Gyr ago.

Given the roughly 4.2–4.3-Gyr earliest date for the first (as distinct from the last) common ancestor, this constrains the lower parts of the bacterial tree to a maximum time length of 500–700 million years (about equal to the length of time from the end of the Precambrian to the present, or less). However, this 'old' view is vigorously challenged by Russell Doolittle *et al.*¹³, who concluded from a study of amino-acid sequences that prokaryotes and eukaryotes all last shared a common ancestor (point A in the figure) roughly 2 Gyr ago, with the divergence of Archaea and Eucarya (point B) about 200 Myr later, roughly 1.8 Gyr ago, a view that is difficult to reconcile with the rocks.

There are two possible explanations why the last common ancestral population seems to have been hyperthermophile. Perhaps a late great impact heated the oceans, so that although the very first life may have lived in cooler water, only those few organisms that could live in very hot water survived to become the ancestors of modern life^{7,6,14}. Certainly, surface organisms would be the most likely to be killed if the top of the ocean vaporized; deeper-living cells would have a better chance of survival. Alternatively, pre-photosynthetic cells may all have lived in hot conditions around early volcanoes. In volcanic hydrothermal systems, heat drives water circulation through the rock: the water becomes charged with a wide variety of chemical species. Where the water emerges from the rock, a diverse biological community can be sustained by the chemical disequilibrium between the hot fluid and the external environment. Modern examples of hydrothermal systems are diverse, ranging from black smokers on mid-ocean ridges to terrestrial hot springs (like those found in Yellowstone National Park in the United States). A hydrothermal origin is consistent with many features of life that appear to be of the deepest antiquity.

Can geological evidence¹⁵ fill in our picture of the setting of the early community of life? Metal-binding proteins, especially those involving iron-sulphur clusters and manganese, and those using



The tree of life, modified after Stetter⁵, from work of Woese *et al.*², Iwabe *et al.*³, and others. Red lines join modern hyperthermophiles. Blue lines link organisms living at lower temperatures. The aeon names are simply illustrative of possible times of branching (indicated as A–D; see text): time increases along the branches, and not necessarily linearly or at the same rate in each branch.

could have survived the early accretionary bombardment of the Earth before, say, about 4.2–4.3 Gyr ago. Even up to about 3.8 Gyr ago or later, the chance that a meteorite impact could vaporize the oceans was significant—the last such impact could have been as early as 4.44 Gyr ago, or as late as 3.8 Gyr ago⁷. Geologically, the isotopic fingerprint of Rubisco-based photosynthesis appears to be present in

copper, zinc or molybdenum, participate in many crucial life processes, such as photosynthesis. To a marine geologist, iron, manganese and sulphur immediately conjure up the image of a black-smoker vent, whereas sulphides of copper, zinc and molybdenum are typical of hydrothermal deposits. Modern biology is very highly skilled at scavenging transition elements from the environment, but the earliest prokaryotes may not have been so able. Perhaps early organisms lived around hydrothermal systems, in environments where metal sulphides were abundant and easily incorporated in biological processes¹⁵—indeed, the problem may have been keeping them out. For example, perhaps infrared phototaxis¹⁶ evolved in organisms living on the mud around deep-water black smokers emitting Fe, Mn and S, a development that preadapted the organisms for the evolution of photosynthesis in shallower, sunlit settings¹⁶. Some Archaean volcanoes erupted unusual lavas known as komatiites, which were comparatively rich in nickel. Perhaps nickel-containing proteins such as urease evolved where nickel sulphide deposits were exposed on komatiite-shield volcanoes.

The importance of the heat-shock proteins¹⁷ in modern organisms may also reflect an early hydrothermal ancestry¹⁵. Although named for their role in protecting organisms against heat shock, today they play a vital part in a wide range of processes (such as photosynthesis) as a result of their role in folding proteins. Their shape, with a characteristic cavity¹⁷, is vaguely reminiscent to a geologist of hydrothermal minerals such as zeolites (faujasite)—perhaps it was inherited from them? Why did early organisms need heat-shock protection? Early hyper-

thermophile organisms living in water near the exits of hydrothermal systems would have been subject to frequent heat shock as currents fluctuated. Is the repair of heat damage merely a function to which heat-shock proteins have been adapted? Any protective device capable of refolding proteins would have been highly advantageous. Did evolution then tinker with early self-repair devices, to create the modern wide array of uses for heat-shock proteins¹⁷?

Perhaps the first living community was an already biologically sophisticated, but relatively undiversified, ancestral population which existed at some time before 3.8 Gyr ago (but after about 4.2 Gyr ago (point A in the figure)—this date depending on the vagaries of when the last large ocean-vaporizing impact took place), living and exchanging genetic information in the 80–110 °C regions of hydrothermal systems, depending on chemotrophy, but not yet capable of photosynthesis, protected by early heat-shock proteins, and accidentally incorporating

locally abundant metals and sulphur into housekeeping proteins. Only later (at point C), when photosynthesis involving CO₂ and H₂O evolved (perhaps 3.5–3.8 Gyr ago⁸) with the help of the heat-shock proteins, was the living community, which by now had diverged into a wide array of types, able fully to escape the hydrothermal systems.

The history of the Eucarya is complex^{18,19}. Primitive Eucarya had no mitochondria; higher Eucarya may have incorporated mitochondria from purple bacteria²⁰. Perhaps the acquisition of chloroplasts (point D) by Eucarya took place at or before 1.9 Gyr ago¹⁹. In summarizing eukaryote history, Knoll¹⁹ concluded with the optimistic hope that geological and molecular evidence might soon converge on a single integrated account. At the moment, the debate is still alive. □

E. G. Nisbet and C. M. R. Fowler are in the Department of Geology, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK.

MATERIALS SCIENCE

The baking of layer cakes

Robert W. Cahn

THIN metallic films, generally made by evaporation onto a substrate, have so many unexpected physical and mechanical properties that they almost rate the status of a distinct state of matter. When two different metals are alternated to form a multilayer, with a repeat distance in the nanometre range, the anomalies multiply. Perhaps the most striking of these anomalies is the giant magnetoresistance that is observed in multilayers such as copper–cobalt or iron–chromium; the electrical resistance can be altered by up to 75 per cent, by applying a magnetic field parallel to the film. In general terms, the mode of alignment of magnetic spins in the transition-metal layers is affected by the spacing introduced by the non-magnetic layers in between, and that affects the resistivity (see ref. 1 for a review).

The use of such multilayers in computer memories is on the way. Other uses of metallic multilayers include reflection of extreme ultraviolet and soft X-rays, and the generation of anomalously high elastic moduli. A review of these properties and a critical treatment of deposition methods can be found in ref. 2.

The efficacy of multilayers in all these functions depends on the sharpness of the metallic interfaces and the constancy of the repeat period. The former, in particular, is affected by taking a multilayer to high temperatures. This has been discussed in a recent paper³ devoted to a study of the effect of annealing at

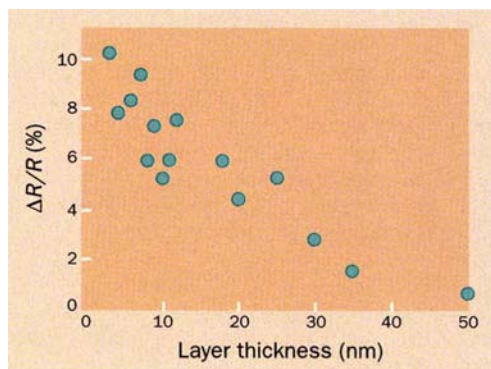
~300 °C on a multilayer of molybdenum and amorphous silicon, which is used for extreme-ultraviolet mirrors. Interdiffusion of the constituents produces a MoSi₂ compound at the interfaces and brings about a large increase of internal stress in the multilayer, which can distort the layer structure. If annealing is carried out at higher temperatures, the interdiffusion can lead to solid-state amorphization, and finally to compound formation throughout the structure, when the constituent metals are mutually reactive⁴. Such effects are of practical concern because high temperatures may be involved either in the deposition process (evaporation, sputtering or chemical vapour deposition) or in subsequent fabrication steps.

There is therefore a clear interest in finding a deposition method that does not raise the temperature of the multilayer as it is built up. One such method is electrodeposition. This can now be done in a single electrolytic bath containing two metallic cations by applying step-changes in the potential difference. With appropriate chemistry, virtually pure metal A or metal B can be made to deposit at each voltage⁵. Jyoko *et al.*⁶ have shown that cobalt–platinum multilayers, of interest for their magneto-optical properties, can be electrodeposited on a single-crystal platinum substrate so as to yield successive layers of both metals in epitaxy (parallel orientation) with the substrate.

- Grogan, D. W. J. *Bacteriol.* **178**, 3207–3211 (1996).
- Woese, C. R. *et al. Proc. Natl Acad. Sci. USA* **87**, 4576–4579 (1990).
- Iwabe, N. *et al. Proc. Natl Acad. Sci. USA* **86**, 9355–9359 (1989).
- Brown, J. R. & Doolittle, W. F. *Proc. Natl Acad. Sci. USA* **92**, 2441–2445 (1995).
- Stetter, K. O. in *Frontiers of Life* (eds Tran Than Van, J. *et al.*) 195–219 (Editions Frontières, Gif-sur-Yvette, France, 1992).
- Forterre, P. *Cell* **85**, 789–792 (1996).
- Sleep, N. *et al. Nature* **342**, 139–142 (1989).
- Schidlowski, M. & Aharon, P. in *Early Organic Evolution: Implications for Mineral and Energy Resources* (eds Schidlowski, M. *et al.*) 147–175 (Springer, Berlin, 1992).
- Awramik, S. in *Early Organic Evolution: Implications for Mineral and Energy Resources* (eds Schidlowski, M. *et al.*) 435–449 (Springer, Berlin, 1992).
- Schopf, J. W. *Science* **260**, 640–646 (1993).
- Lowe, D. R. *Geology* **22**, 387–390 (1994).
- Wilks, M. E. & Nisbet, E. G. *Can. J. Earth Sci.* **22**, 792–799 (1985).
- Doolittle, R. F. *et al. Science* **271**, 470–477 (1996).
- Gogarten-Boekels, M. *et al. Orig. Life Evol. Biosp.* **25**, 251–264 (1995).
- Nisbet, E. G. in *Early Precambrian Processes* (eds Coward, M. P. & Ries, A. C.) 27–51 (Spec. Publ. Geol. Soc. Lond. 95, 1995).
- Nisbet, E. G. *et al. Nature* **373**, 479–480 (1995).
- Hunt, J. F. *et al. Nature* **379**, 37–45 (1996).
- Edlind, T. D. *et al. Mol. Phylogenet. Evol.* **5**, 359–367 (1996).
- Knoll, A. H. *Science* **256**, 622–627 (1992).
- Blackstone, N. W. *Evolution* **49**, 785–796 (1995).

More recently, a Russian team⁷ electro-deposited a copper-nickel multilayer on a single-crystal gallium arsenide substrate, and again found the layers to be in epitaxy, allowing more uniform and reproducible growth. Because of the mismatch between the lattice constants of GaAs and the metals, intense strains are generated in the initial metallic layers, but they attenuate as further layers are added.

Undoubtedly, the most piquant use of



The change in resistance R due to an applied magnetic field of nanowires made of alternating layers of copper and cobalt. The effect increases with decreasing thickness (from ref. 8).

the electrodeposition approach to making multilayers is a recent study by two Swiss physicists⁸. Doudin and Ansermet wished to make fine wires of multilayered construction (long stacks of thin disks, in other words) so as to measure the giant magnetoresistance (GMR) along the wires while a field was applied normal to the wires. They achieved this seemingly impossible task by using a thin polymeric membrane with ultra-fine holes 0.1 μm in diameter, coating one side of the membrane with a gold electrode, and plating copper and cobalt from a complex plating bath through the holes. The membranes (six micrometres thick) are made by irradiating polycarbonate films with heavy ions and etching through the films along the radiation-damage tracks^{9,10}, a technique originally developed in the 1950s for quite different purposes. By making thousands of layers, Doudin and Ansermet were able to achieve unusually high resistance values in these fine wires. The figure shows how the magnetoresistance effect changed with reduction of the layer thickness, as expected.

As an aside, the authors refer to a number of applications of track-etched membranes by scientists with very diverse interests. The first such use, in 1962, was to filter cancer cells from blood by using such membranes as ultrafilters¹⁰; that is why such membranes are commercially available today.

Another effect of annealing, this time at temperatures up to 270 °C, is to reduce by a third the magnetoresistance of copper-cobalt multilayers made by

sputtering¹¹. The variation of GMR with magnetization was consistent with the notion that changes in GMR are due to changes in the antiferromagnetically aligned volume fraction. In this study, perhaps because of the modest temperatures used, there was no evidence of any changes in the interfaces between the layers.

One aspect of multilayers that has given rise to some dispute is the so-called supermodulus effect, which I reported in these columns a decade ago¹² when it had been newly discovered. Large changes in flexural elastic modulus (stiffness) were observed in copper-nickel multilayers, with the maximum effect around two-nanometre periodicity. I presented the rival models advanced to explain this curious effect, but some years later Kumar *et al.*¹³ found themselves unable to reproduce the supermodulus effect in copper-cobalt, and interest waned. More recently still, a Japanese team¹⁴ used improved methods in an attempt to re-establish the effect. They had single-crystal GaAs as a substrate as did the Russians, but instead of electrodeposition, they used molecular-beam epitaxy to deposit high-quality multilayers in parallel orientation. They were critical of the direct mechanical methods that had been used earlier to measure flexural moduli, and instead measured surface acoustic waves to obtain an indirect but precise measure of elasticity. They found an increase in the elastic constant of around 60%, so it seems that the supermodulus effect has been rescued. Now, perhaps, applications will be found. The very international nature of the research effort on multilayers suggests that this may not take long. □

Robert W. Cahn is in the Department of Materials Science and Metallurgy, University of Cambridge, Pembroke Street, Cambridge CB2 3QZ, UK.

1. Howson, M. A. *Contemp. Phys.* **35**, 347–359 (1994).
2. Greer, A. L. & Somekh, R. E. in *Processing of Metals and Alloys* (ed. Cahn, R. W.) 329 (VCH, Weinheim, 1991).
3. Kassner, M. E. *et al. J. Mater. Sci.* **31**, 2291–2299 (1996).
4. Sinclair, R. & Konno, T. *J. Magn. Magn. Mater.* **126**, 108–112 (1993).
5. Ros, J. R. *et al. in Processing of Metals and Alloys* (ed. Cahn, R. W.) 481 (VCH, Weinheim, 1991).
6. Jyoko, Y. *et al. J. Magn. Magn. Mater.* **126**, 52–54 (1993).
7. Demenko, A. V. *et al. J. Mater. Synth. Processing* **3**, 303–306 (1995).
8. Doudin, B. & Ansermet, J.-Ph. *Nanostructured Mater.* **6**, 521–524 (1995).
9. Martin, C. R. *Adv. Mater.* **3**, 457–459 (1991).
10. Fleischer, R. L. *et al. Science* **143**, 249 (1964).
11. Hall, M. J. *et al. Thin Solid Films* **275**, 195–198 (1996).
12. Cahn, R. W. *Nature* **324**, 108–109 (1986).
13. Kumar, S. *et al. Phys. Rev. B* **44**, 5905 (1991).
14. Sakaue, K. *et al. J. Magn. Magn. Mater.* **126**, 207–209 (1993).

Travel by cola

DAEDALUS once proposed a sea-going hovercraft pressurized, not by air pumped into it from above, but by evaporation of the water below. Its hot underside was to warm up, and thus increase the vapour pressure of the water beneath it. To obtain 0.1 atmospheres of pressure, the water surface would have to be heated by about 30 °C during the passage of the craft.

Sadly, this rate of heat transfer proved unfeasible. But Daedalus now has a new twist. He points out that carbonated soft drinks are pressurized to several atmospheres. Yet when you pour them out, most of the gas stays dissolved as a supersaturated solution. A hovercraft travelling over a lake or canal filled with such a drink could, in principle, exploit the massive vapour pressure of its surface.

In this connection, Daedalus recalls that the evaporation of water from a reservoir can be impeded by covering its surface with a monolayer of cetyl alcohol. Such monolayers flow over a water surface as a sort of two-dimensional liquid. By compacting them between barriers, they can be compressed to the equivalent of hundreds of atmospheres. So Daedalus reckons that even the most ferociously carbonated soda water would hold its gas stably if it were covered with a compact monolayer of cetyl alcohol, oleic acid, or other film-forming surfactant.

Imagine, says Daedalus, a lake or canal full of soda water loaded with many atmospheres of carbon dioxide, kept in solution by a surface monolayer. The 'carbocraft' comes along, supported on the cushion of compressed carbon dioxide beneath it. This cushion presses on the water surface, indenting it into a depression beneath the flying vehicle. The deformation, of course, increases the surface area of the water. The monolayer cannot stretch to cover the extra area; it separates to uncover free water surface, from which gas evaporates at many atmospheres. The craft is thus levitated on a cushion of the gas released by its passage. When it has passed, the monolayer closes and heals again, and no further gas is lost.

The carbocraft will be swift, silent and simple. Daedalus hopes that it can be propelled and steered by a jet efflux of the gas it harvests from the water. It could ply its trade on special canals, kept supersaturated by a pipe along the bottom releasing carbon dioxide under pressure. But it would be most at home on those volcanic lakes that are naturally supersaturated by some underground source of geological carbon dioxide.

David Jones

Endohedral fullerene production

SIR — The properties of metal-containing endohedral fullerenes (in which the metal is trapped inside the carbon cage) might be tailored to give interesting electronic materials, such as superconductors¹, that are less sensitive to air than the presently available fullerene-based alkali intercalated materials². We have discovered a method for efficient production of macroscopic amounts of endohedral alkali-metal fullerenes, which should be generally applicable to other endohedral species.

Although there is considerable evidence for endohedral fullerenes^{3–8}, it is difficult and time-consuming to produce and isolate sufficient quantities to investigate their properties. The mass spectra of sublimed soot obtained from either the standard arc vaporization⁹ or high-temperature laser vaporization¹⁰ of graphite doped with the appropriate metal oxide or salt yield evidence for endohedral species.

However, it has been possible chromatographically to isolate only $M@C_{82}$, where M is a Group III metal (La, Sc, Y) or a lanthanide and the @ symbol designates endohedral species, and to a lesser extent $Sc_2@C_{2n}$, in amounts large enough for further studies^{11,12}. Heating C_{60} to 600 °C under high pressure (2,500 atm) of a rare gas for a few hours¹³ yields a small fraction of a per cent of C_{60} molecules that have captured a rare-gas atom (0.1% for He, 0.008% for Xe)¹⁴.

In our method, we repeatedly expose monolayers of C_{60} to an intense beam of alkali ions at an energy chosen so that the ions can penetrate the carbon cage but cannot destroy it. In this way we can build up a film of fullerenes many nanometres thick in which a substantial percentage of the C_{60} molecules contains an alkali-metal ion. Gas-phase collision experiments⁷ provide values for the energetic thresholds for capture of alkali ions: approximately 6 eV for Li^+ , increasing to approximately 40 eV for K^+ .

At collision energies beyond 30 eV in these gas-phase experiments, the C_{60} cage fragments, leading to a rapid drop in the intensity of the unfragmented endohedral species and increasing intensities of $M@C_{60-2n}^+$ fragments. Because in our experiments we are irradiating layers of C_{60} with the ion beam, we can dissipate the vibrational energy transferred to the cage on capture of the ion to the bulk and thus stabilize the endohedral species and avoid fragmentation of the cage.

The figure (a) shows a laser-desorption mass spectrum obtained from a 300-Å-thick film of C_{60} exposed to a Li^+ beam of 30-eV kinetic energy after the deposition

of each monolayer. We estimate the $Li^+:C_{60}$ ratio during deposition to be 6:1, which gives the best capture conditions. The very clear signal corresponding to $Li@C_{60}^+$ has an intensity of 27% of the C_{60}^+ ion peak. The very small amount of C_{58}^+ also present in the spectrum is produced by laser desorption. There is no evidence for fragmentation of the deposited fullerenes during ion irradiation at this ion-beam energy. The measured spectra remained unchanged after exposing the film to air for a period of months, in contrast to the well-known rapid degradation of thermally doped alkali-intercalated fullerene materials. Under the conditions used to obtain this sample, we can deposit approximately 15 µg endohedral $Li@C_{60}$ per hour. This should be further improved by optimization.

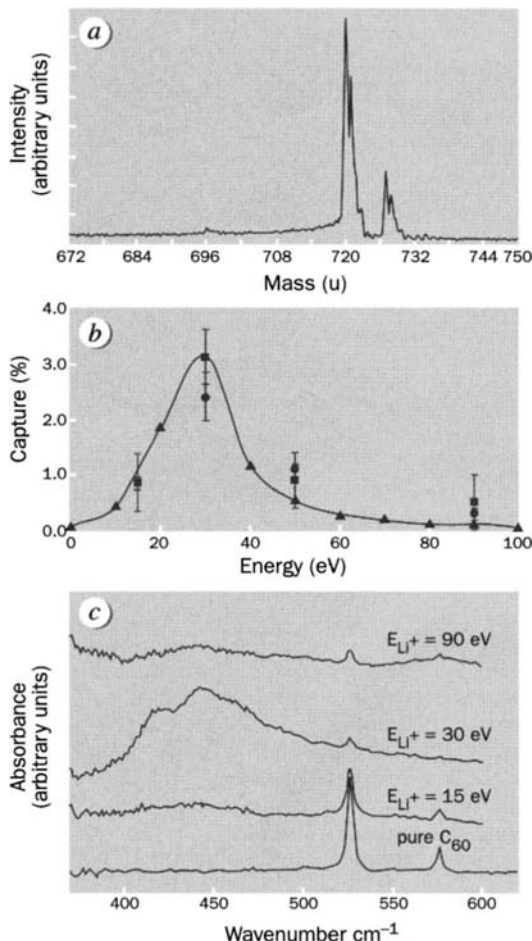
We investigated the collision-energy dependence of the process for a $Li^+:C_{60}$ ratio of 1:1 during production. Although the intensity of the endohedral signal is significantly smaller than with the higher ion-beam intensity used in figure a it is still a considerable 3%. A strong indication that the species produced are indeed endohedral is obtained from the identical

results for positively and negatively charged laser-desorbed ions in the figure (b). Using the relative cross-section for the sum of all endohedral species (including fragments) from ref. 7, we obtain good agreement with their collision-energy dependence of endohedral formation, confirming our supposition that the endohedral species can be stabilized by dissipation of the vibrational energy to the bulk or underlying substrate.

There have been several theoretical predictions for infrared spectra of endohedral fullerenes^{15–17}. Vibrational and rolling motion of the captured ion in the fullerene cage produces a modulation of the molecular dipole moment, leading to the absorption and emission of radiation in the microwave and far-infrared regions. The fundamental vibration-rotation band for Li^+ , corresponding to a rattling motion of the ion within the cage, has been predicted¹⁵ to be fairly strong and to show a P branch lying between 300 and 350 cm^{-1} and an R branch in the range 350–500 cm^{-1} with the maximum at around 375 cm^{-1} for a temperature of 25 °C.

We carried out infrared absorption spectroscopy in this range on a series of $Li@C_{60}$ films produced with different ion impact energies (15–90 eV) (c in the figure). We see a strong signal in the range

a, Mass spectrum of positively charged ions produced by pulsed excimer laser desorption/ionisation of a film containing endohedral $Li@C_{60}$. Multiple peaks are due to the presence of the ^{13}C isotope. Film was produced by repeatedly exposing monolayers of C_{60} to an intense Li^+ beam ($Li^+:C_{60}$ ratio of 6:1) at an energy of 30 eV. b, Percentage of unfragmented $Li@C_{60}$ observed with laser desorption mass spectrometry of the prepared films as a function of ion collision energy with a $Li^+:C_{60}$ ratio of 1:1. Squares, positively charged ions, circles: negatively charged ions; triangles: results of gas phase collision experiments in ref. 7 summed over all non-fragmented and fragmented species and normalized to the positive ion laser desorption results at an ion energy of 30 eV. The full line drawn through the data points of ref. 7 is to guide the eye. c, Infrared absorption spectra of films produced with ions of different energies. The spectrum from a pure C_{60} film is shown for comparison. A strong signal due to the fundamental vibration-rotation bands of the Li^+ inside the fullerene cage can be observed for the film produced with an ion energy of 30 eV.



400–550 cm^{-1} for films produced with an ion energy of 30 eV (the energy that produced the maximum endohedral signal in the laser-desorption mass-spectrometry results). The double maximum that we observe shows close similarities with the predicted P and R branches but is shifted by about 50–75 cm^{-1} to the blue, indicating that the endohedral potential is slightly deeper and narrower than predicted theoretically.

As our method produces films that contain only the particular fullerene deposited and its endohedral version in a ratio of around 3:1 (for Li), we can investigate the properties of the endohedral material without further purification. Similar results using Na^+ , K^+ and Rb^+ ion beams show that the method can be extended to larger ions and might also be applicable to non-alkali-metal systems.

R. Tellmann, N. Krawez, S.-H. Lin*, I. V. Hertel & E. B. Campbell

Max-Born-Institut für Nichtlineare Optik und Kurzzeitspektroskopie,
Postfach 1107,
D-12474 Berlin, Germany

*Permanent address: Institute of Nuclear Research, Academia Sinica, PO Box 800-204, Shanghai 201800, China

- Whitehouse, D. B. & Buckingham, A. D. *Chem. Phys. Lett.* **207**, 332–338 (1993).
- Fleming, R. M., Zhou, O., Murphy, D. W. & Siegrist, T. in *Progress in Fullerene Research* (eds Kuzmany, H. et al.) 211–216 (World Scientific, Singapore 1994).
- Heath, J. R. et al. *J. Am. Chem. Soc.* **107**, 7779–7780 (1985).
- Weiss, F. D. et al. *J. Am. Chem. Soc.* **110**, 4464–4465 (1988).
- Wieske, T. et al. *Angew. Chem. Int. Ed. Engl.* **30**, 884–886 (1991).
- Sprang, H., Mahlkow, A. & Campbell, E. B. *Chem. Phys. Lett.* **227**, 91–97 (1994).
- Wan, Z., Christian, J. F., Basir, Y. & Anderson, S. L. *J. Chem. Phys.* **99**, 5858–5870 (1993).
- Gillan, E. G. et al. *J. Phys. Chem.* **96**, 6869–6871 (1992).
- Bethune, D. S. et al. *Nature* **366**, 123–128 (1993).
- Chai, Y. et al. *J. Phys. Chem.* **95**, 7564–7568 (1991).
- Kikuchi, K. et al. *Chem. Phys. Lett.* **216**, 67–71 (1993).
- Shinohara, H. et al. *J. Phys. Chem.* **97**, 4259–4261 (1993).
- Saunders, M. et al. *Science* **259**, 1428–1430 (1993).
- Saunders, M. et al. *J. Am. Chem. Soc.* **116**, 2193–2194 (1994).
- Joslin, C. G. et al. *Chem. Phys. Lett.* **208**, 86–92 (1993).
- Li, Y. S. & Tomanek, D. *Chem. Phys. Lett.* **221**, 453–458 (1994).
- Van Cleef, G. W., Renkes, G. D. & Coe, J. V. *J. Chem. Phys.* **98**, 860–864 (1993).

Genome analysis

SIR — The recent commentary from Venter, Smith and Hood¹ proposed a simplified approach to sequencing the human genome. Many parts of this proposal are very attractive and make good use of the dispersed facilities that exist internationally for small, large and massive scale DNA sequence determination.

The ultimate use of the “sequence-tagged connector” (STC) approach depends very much on the quality of the library that is to act as a shared public resource; the authors do not discuss this problem in detail. They cite two studies^{2,3}

to argue that bacterial artificial chromosome (BAC) clones “seem to represent human DNA far more faithfully than their YAC (yeast artificial chromosome) or cosmid counterparts”. Both refs 2 and 3 show that DNA is stable in BAC clones that are subject to serial propagation. The basis of instability of cosmids under these circumstances has been discussed by several workers⁴ but this misses a key point: the technical problem has been that regions of DNA simply do not appear in properly grown cosmid (and YAC) libraries, not that a high proportion of deleted cosmids are found in such cases (although this is sometimes found in badly handled libraries). This suggests that the difficulty is not simply in the intrinsic instability of high-copy cosmid-replication systems. It is still unclear that BACs are any more successful with the problematic regions because, by definition, these regions have not been isolated from human DNA.

A second issue that also needs to be addressed is the actual distribution of ends of DNA molecules generated by random shear (or partial digestion) of total genomic DNA. Very little is known about the detailed distribution of end-sequences under these experimental conditions, particularly after the necessarily gentle procedures for isolation of large DNAs have been used. In most library constructions this is not normally a problem but in the STC proposal it becomes a significant issue: clustering of end points or under-representation of regions would generate gaps in STC coverage. Of course, these can be identified by the developing physical mapping reagents, which is precisely the role that these maps have always occupied in ‘traditional’ descriptions of the genome project.

When they first appeared, YAC libraries were widely held to overcome many of the difficulties associated with cosmids: it is only now that we have a clear understanding of their deficiencies. Indeed, Venter et al. point out that in the T-cell receptor region, 1 BAC clone in 17 has a deletion, suggesting 5–6% of BAC clones could be rearranged.

The reliance of the STC proposal on a single library has obvious advantages, but does require that the properties of the target library be very well understood. It is not clear that any single cloning vector is yet understood in these terms. As a consequence, the undeniable cost effectiveness, ready availability and ease of resource sharing of the library underpinning the STC proposal, should not be allowed to obscure the heavy reliance of this approach on an untested quantity, that of clone distribution and stability in a rigorous sense. It would be a mistake to assume, once again, that technical advances can be frozen by selection of exclusive approaches. In genome analysis,

flexibility of resources as well as approach must remain a key strategy.

Peter Little

Department of Biochemistry
Imperial College of Science, Technology
and Medicine,
London SW7 2AZ, UK

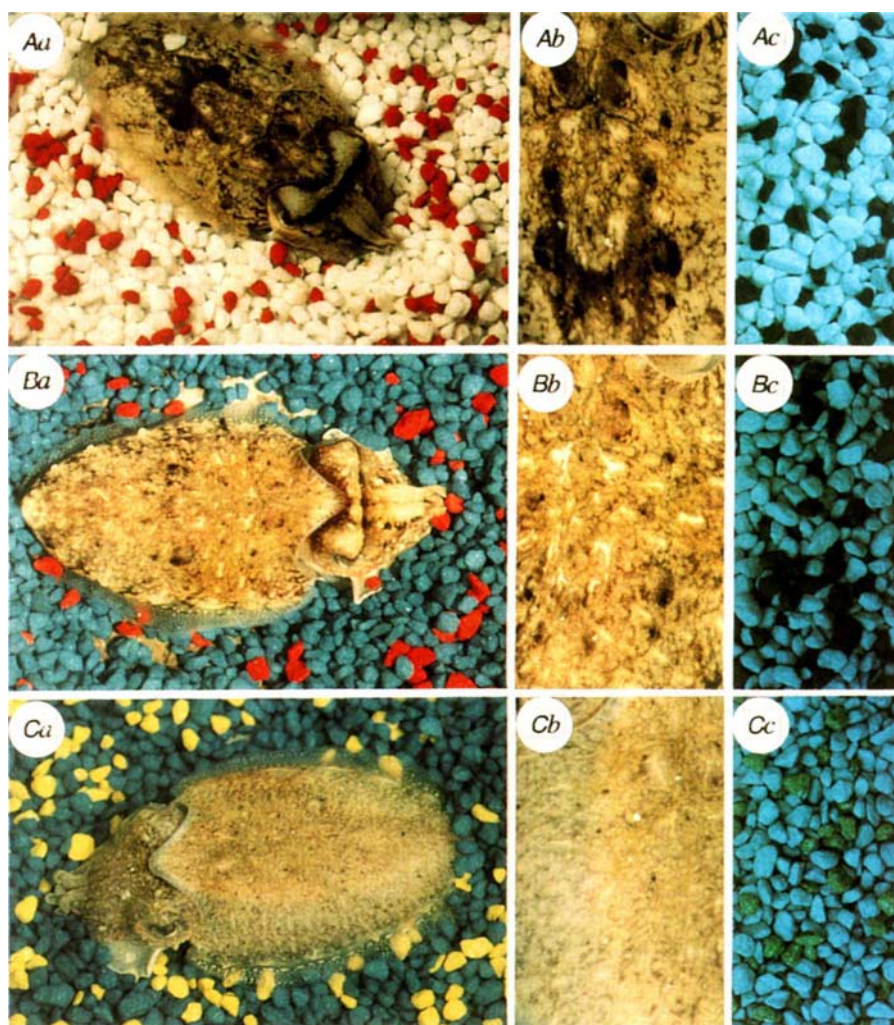
- Venter, J. C., Smith, H. O. & Hood, L. *Nature* **381**, 364–366 (1996).
- Kim, U. J. et al. *Nucleic Acids Res.* **20**, 1083–1085 (1992).
- Shizuya, H. et al. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8794–8797 (1992).
- Little P. F. R. & Cross, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3159–3163 (1985).

Colour-blind camouflage

SIR — Cuttlefish are remarkable for their powers of camouflage, which is in part due to their ability to generate disruptive patterns in the skin, using neurally controlled chromatophores. We show here, however, that cuttlefish produce patterns on variegated backgrounds only if these contain appreciable differences in intensity: differences in wavelength are not responded to. This result may seem counterintuitive, but it supports other evidence that cuttlefish (like octopuses) are colour blind.

Although it is well known that cephalopods that live on the bottom of the sea use chromatophores to camouflage themselves on various backgrounds, it is not generally realized that like most cephalopods (with the notable exception of the firefly squid, *Watasenia*^{1,2}) they are almost certainly colour-blind^{3–5}. How do such animals match their background so well? One theory^{6,7} has emphasized that they achieve a degree of “general colour resemblance”⁸ with iridophores and leucophores, which can reflect the predominant wavelengths in the immediate environment. Yet effective camouflage does not depend on general colour resemblance alone. One powerful and widespread technique used by animals for crypsis is patterning, especially the various forms of “disruptive” patterns⁸ that break up the overall form of the body. Patterning in cephalopods is effected by the chromatophores, and because these are neurally controlled the animal can use them to generate, as appropriate, a range of finely graded patterns in its skin, from uniform, through stipples and mottles to bolds and disruptives⁹.

Cuttlefish (*Sepia officinalis*) have numerous, small chromatophores containing either yellow, orange-red or dark brown pigment granules⁹, and they have large eyes with a single visual pigment (λ_{max} 492 nm; ref. 10). We tested their patterning responses to a series of specially designed backgrounds. We placed individuals in small tanks containing a gravel substrate, and once they had settled we



◀ Fig. 1 Aa, Whole animal (mantle length about 100 mm) photographed in daylight on a prepared background — red gravel on white; Ab, detail of mantle skin of the same animal (magnification X 2); Ac, the same background photographed through a green interference filter ($\lambda_{\max}$ 490). Kodachrome 64. B, As A, but with red gravel on blue. C, As A, but with yellow gravel on blue.

photographed them in daylight, with and without a 'green' interference filter ($\lambda_{\max}$ 490). We used white, blue, green, yellow and red gravels, singly and in various paired combinations, with a few stones of one colour evenly scattered on a background of the other.

Examples of the responses of cuttlefish to three combinations of coloured

gravel are shown in Fig. 1, which shows that the animals are not matching their body colour to that of the gravel background. This is even more obvious when the mantle skin is examined in close-up. Yet the photographs also show that the patterning response to the three backgrounds differs considerably. A cuttlefish tends to become uniformly dark on red

gravel, and uniformly pale on white, but when confronted with red gravel on white (Fig. 1Aa) it produces a bold, coarse patterning, with 'dark mottle' on the mantle and 'disruptive' on the head and arms. On a background of red gravel on blue, however, it produces a much less bold pattern: 'light mottle' (Fig. 1Ba); and on a background of yellow gravel on blue it shows an extremely fine, almost uniform pattern: 'stipple' (Fig. 1Ca). The lack of patterning with yellow on blue gravels, which make such a striking background for humans, may seem strange but is understandable if we consider the contrast of the gravels as they would appear to an animal with a single visual pigment peaking in the green (Fig. 2). It can be seen now that the yellow and blue gravels must appear very similar (Fig. 1Cc); the red gravel is substantially darker than the blue, however (Fig. 1Bc), and the red contrasts markedly with the white (Fig. 1Ac).

Our photographs provide evidence that cuttlefish do not respond to different wavelengths in the substrate as they regulate their chromatophores. However, where they perceive different intensities in the background they generate patterns in the skin: the higher the contrast in the background the bolder the pattern. Clearly, an animal with only a single visual pigment can produce patterns for crypsis where there are intensity differences in the background. This demonstration should finally lay to rest doubts about how cephalopods can camouflage themselves despite being colour blind.

N. J. Marshall

Sussex Centre for Neuroscience,
School of Biological Sciences,
University of Sussex,
Brighton BN1 9QG, UK

J. B. Messenger*

Department of Animal & Plant Sciences,
University of Sheffield,
Sheffield S10 2TN, UK

*To whom correspondence should be addressed.

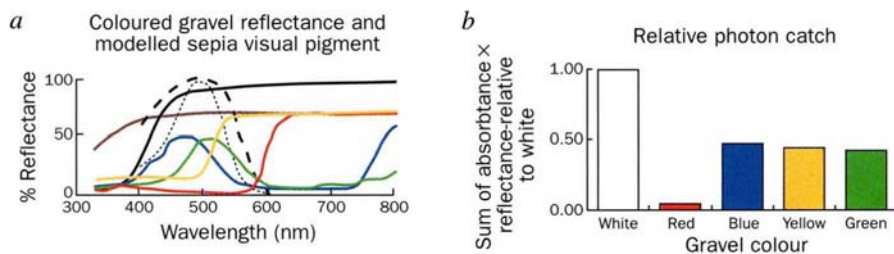


Fig. 2a, Spectral characteristics of gravels used (light grey line, white) and *Sepia* lens transmittance. Also plotted are a visual pigment nomogram curve (kindly supplied by Gary Bernard) for *Sepia* visual pigment (at $\lambda_{\max}$ 492 nm), and calculated absorbance of a photoreceptor based on $0.008 \mu\text{m}^{-1}$ unit absorbance and a photoreceptor length of $200 \mu\text{m}$. Measurements made with "Sub-Spec", a custom-made spot spectrophotometer (Oriel Instruments/Andor Technology), an underwater multi-purpose CCD spectrograph, a xenon arc lamp and a "Spectralon" white standard as reference. b, Calculated photon catch of a *Sepia* photoreceptor looking at each gravel type relative to white gravel. The area of each histogram block is the sum of the products of each gravel reflectance curve and absorbance curve at 1-nm intervals. The lens is assumed to act as a perfect 400-nm cut-off filter so data were summed over the range 400–600 nm only. Blue, yellow and green gravels have very similar relative brightnesses to the presumed monochromatic *Sepia* visual system. Experiments were carried out at the Marine Biological Association Laboratory, Plymouth PL1 2PB, UK.

1. Seidou, M. et al. *J. Comp. Physiol. A* **166**, 769–773 (1990).
2. Minchinomae, M., Masuda, H., Seidou, M. & Kito, Y. *J. Exp. Biol.* **193**, 1–12 (1994).
3. Messenger, J. B., Wilson, A. P. & Hedge, A. J. *Exp. Biol.* **59**, 77–94 (1973).
4. Roffe, T. *Vis. Res.* **15**, 353–356 (1975).
5. Messenger, J. B. *J. Exp. Biol.* **70**, 49–55 (1977).
6. Messenger, J. B. *J. Zool.* **174**, 387–395 (1974).
7. Messenger, J. B. *Endeavour* **3**, 92–98 (1979).
8. Cott, H. B. *Adaptive Coloration in Animals* (Methuen, London, 1940).
9. Hanlon, R. T. & Messenger, J. B. *Phil. Trans. R. Soc. B* **320**, 437–487 (1988).
10. Brown, P. K. & Brown, P. S. *Nature* **182**, 1288–1290 (1958).

Cyclin encoded by KS herpesvirus

SIR— Kaposi's sarcoma(KS)associated herpesvirus (KSHV or HHV8) has been strongly implicated in the development of Kaposi's sarcoma and primary effusion lymphomas^{1,2}. This herpesvirus was initially described in Kaposi's sarcoma associated with AIDS patients¹, but has since been found in all epidemiological forms of Kaposi's sarcoma³⁻⁵. KSHV is a gamma-herpesvirus, similar to other oncogenic herpesviruses such as the New World monkey virus, herpesvirus saimiri, and Epstein-Barr virus⁶. Genomic sequencing of KSHV has thus far failed to identify sequence similarity to genes of Epstein-Barr virus or herpesvirus saimiri which are known to have important oncogenic functions (P. S. M., unpublished data). However, we report here that KSHV contains an open reading frame (ORF 72) with sequence similarity to cellular cyclins, in particular the D-type cyclins⁷ (a in the figure). (Herpesvirus saimiri also has an open reading frame with similarity to cyclins^{8,9}.)

Various lines of evidence suggest that deregulated expression of cellular D-type cyclins is linked to the development of tumours in humans¹⁰. Cyclin D proteins are

regulatory subunits which activate cellular kinases (CDKs) to phosphorylate check-point molecules such as the retinoblastoma-tumour suppressor protein (RB)¹¹. The similarity of KSHV ORF 72 to cellular D-type cyclins suggests that its encoded product, KSHV-cyclin, could also stimulate such kinases resulting in their unlicensed activation and, consequently, cause deregulation of cellular proliferation inherent to KSHV associated diseases.

To determine whether KSHV-cyclin could activate kinases and perturb control of cell growth in a manner analogous to aberrant expression of D-type cyclins, we engineered an epitope-tagged (c-myc epitope) version of KSHV ORF 72 driven by the cytomegalovirus immediate early promoter. On transfection into COS-1 cells, this construct gives rise to expression of a polypeptide of relative molecular mass 28,000 that, like the D-type cyclins, is localized to the cell nucleus. The KSHV-cyclin protein is associated with kinase activity capable of phosphorylating RB *in vitro* at authentic sites, including those previously shown to be a hallmark of RB inactivation¹² (b in the figure). This supports the hypothesis

that KSHV-cyclin associated kinases could mediate the functional inactivation of RB and thereby overcome the cell-cycle arrest imposed by RB.

To test this possibility directly we co-expressed the KSHV-cyclin together with RB in SAOS-2 osteosarcoma cells which, due to the mutation of both RB alleles, lack expression of endogenous RB¹³. On transfection of wild-type RB, these cells stop growing, resulting in a spreading and senescent-appearing morphology¹⁴. Co-expression of KSHV-cyclin in these cells abrogates this response, as previously noted with cellular cyclins. Moreover, the RB detected in cell lysates from such co-transfected cells shows a retarded migration characteristic of hyperphosphorylated, inactive RB (c in the figure).

Taken together, our findings imply that the ectopic expression of KSHV-cyclin in latently infected host cells can inappropriately activate kinases whose activity normally requires cellular cyclins. This in turn can allow such cells to overcome growth-restriction checkpoints, such as that imposed by RB. Although direct evidence for a transforming role of KSHV-cyclin has yet to be established, we have found KSHV-cyclin transcripts in three out of three primary Kaposi's sarcoma biopsies of different aetiology and in a primary effusion lymphoma, indicating a role for KSHV-cyclin in these tumours. The ability of KSHV-cyclin to overcome RB-mediated cell cycle arrest in SAOS2 cells provides direct evidence for a mechanism by which this virus could contribute to tumour development.

Yuan Chang & Patrick S. Moore

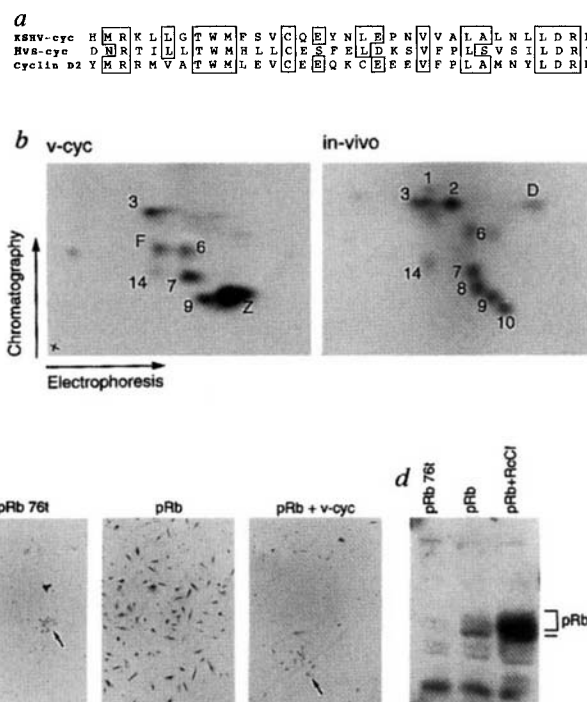
Division of Epidemiology,
School of Public Health and Department of
Pathology,
College of Physicians and Surgeons,
Columbia University, New York,
New York 10032, USA

**Simon J. Talbot, Chris H. Boshoff,
Tamara Zarkowska, Diana Godden-Kent,
Hugh Paterson, Robin A. Weiss,
Sibylle Mitnacht***

CRC Centre for Cell and Molecular Biology
and Virology Laboratory,
Institute of Cancer Research,
Chester Beatty Laboratories,
London SW3 6JB, UK

* To whom correspondence should be addressed.

a, Amino-acid sequence alignment of the putative 'cyclin box' of KSHV-cyclin (residues 49-82) with HVS cyclin (residues 50-83) and human cyclin D2 (residues 54-87). Identical residues to KSHV-cyclin are boxed. b, Two-dimensional tryptic peptide pattern of full-length RB phosphorylated by KSHV-cyclin immunocomplex *in vitro* (v-cyc) and of natural RB immunopurified with anti-RB antiserum from ³²P labelled human cells (*in vivo*) demonstrating that authentic sites are phosphorylated by the KSHV-cyclin associated kinase¹². c, SAOS-2 senescence assay. Photomicrograph of transfected cell monolayers after puromycin selection for 12 days are shown. Monolayers of senescent cells with enlarged cell bodies are seen when cells are transfected with wild-type RB (middle panel) but not in cells transfected with RB 76t mutant (left panel) or when wild-type RB is transfected together with KSHV-cyclin. Arrowheads in the left and right-hand panels denote colonies of puromycin resistant, normal size cells which form under the latter two conditions. d, Western blot analysis of RB protein in SAOS-2 cell lysates prepared 4 days after transfection showing retarded migration of RB (characteristic of inactive RB) in the KSHV-cyclin transfected sample (RB + RCMV.v-cyc). Methods (available in full from the authors): SAOS-2 cells were transfected with expression plasmids encoding a non-functional pRB mutant in which a termination codon was introduced at amino-acid 76 (RB 76t), wild-type RB (RB) or a mixture of wild-type RB and KSHV-cyclin (v-cyc). Co-transfection with a puromycin resistance plasmid was performed.



1. Chang, Y. *et al.* *Science* **266**, 1865-1869 (1994).
2. Cesarman, E. *et al.* *New Eng. J. Med.* **332**, 1186-1191 (1995).
3. Boshoff, C. *et al.* *Nature Med.* **1**, 1274-1278 (1995).
4. Boshoff, C. *et al.* *Lancet* **345**, 1043-1044 (1995).
5. Moore, P. S. & Chang, Y. *New Eng. J. Med.* **332**, 1181-1185 (1995).
6. Moore, P. S. *et al.* *J. Virol.* **70**, 549-558 (1996).
7. Cesarman, E., *et al.* *J. Virol.* (in the press).
8. Nicholas, J., Cameron, K. R. & Honess, R. W. *Nature* **355**, 362-365 (1992).
9. Jung, J. U., Stager, M. & Desrosiers, R. C. *Mol. Cell. Biol.* **14**, 7235-7244 (1994).
10. Jacks, T. & Weinberg, R. A. *Nature* **381**, 643-644 (1996).
11. Sherr, C. J. *Trends Biochem. Sci.* **20**, 187-190 (1995).
12. Mitnacht, S. *et al.* *EMBO J.* **13**, 118-127 (1994).
13. Shew, J. Y. *et al.* *Oncogene Res.* **4**, 205-214 (1989).
14. Huang, H. J. *et al.* *Science* **242**, 1563-1566 (1988).

America's greatest gift

Ryan J. Huxtable

The True History of Chocolate. By Sophie D. Coe and Michael D. Coe. *Thames and Hudson*: 1996. Pp. 280. £16.95, \$27.50.

It is difficult to recreate the impact that the discovery of the Americas had on the European psyche. The tight hermetic continent of the Middle Ages was reborn into a seemingly illimitable universe that reshaped perception of both the inner and the outer world. This period is well-named the Renaissance. "O my America! my new-found-land", moans the ecstatic lover of Donne's poem "Going to Bed", equating the exploration of his mistress to the geographical discoveries of his day, while Marvel reflectively muses on "Where the remote Bermudas ride" while attempting a poetic seduction. Although Shakespeare's Caliban responds with "O brave new world that has such people in't" on meeting his first Europeans — a pair of rascally drunkards — it is in fact the Europeans who have found the brave new world, with its apparent cornucopia of drugs (guaiac, cinchona), foods (corn, potatoes, chilli peppers), Orinoco gold and botanical wonders such as the misnamed American aloe (actually an agave) of Dickens's *Martin Chuzzlewit* ("You are the American aloe of the human race, my dear Chiv", said Mr Tigg, "which only blooms once in a hundred years!").

And then, of course, there was chocolate, the greatest gift of the New World to the Old. Louis Lémery (1704) claimed that chocolate was without doubt the best thing that had come out of America, although he qualified this by adding with the wilful blindness of the early European explorers "next to gold and silver". The lunatic search for gold led Sir Walter Raleigh to the edge of insanity and then beyond the sword's edge in the Tower of London. It involved the extermination of many thousands of Amerindians, and, in the absence of economic understanding, to inflation in the importing countries, with the Spanish unable to comprehend why the price of eggs had increased so much when the nation had supposedly become so much wealthier.

Chocolate was prepared by a complicated process from the seeds of a tree that produced flowers from, mysteriously to European eyes, its trunk rather than its branches. This source of the most sinful of foods, a prelapsarian gift of the gods, was appropriately named *Theobroma cacao* by Linné. The genus name derives from the Greek 'food of the Gods', whereas the species name derives from an indigenous term. The authors of *The True History of Chocolate* comment that Linné found this

New World name "barbaric". This is no doubt because of its similarity to the Greek word *kakkáo* (defecate). (Linné presumably knew that the literal meaning of barbaric is the inability to speak Greek.)

The book traces how the bitter chocolate drink, blended with substances as diverse as chilli, achiote or vanilla and used ritualistically by the Aztecs, transmuted itself into a sweetened solid consumed in



Early eighteenth-century tile panel showing a *chocolatada* (chocolate party) in Valencia, Spain.

vast quantities by modern western populations. Despite sustaining a loyal clientele and having a myriad specialist magazines devoted to it, chocolate consumption is still largely a western addiction, having only marginally penetrated the Arab and Far Eastern worlds (the 'explanation' given for this is "cultural conservatism").

Half of the book is devoted to the Mesoamerican history (or prehistory) of chocolate. This expensive commodity was used by the Aztecs, Mayans and Olmecs, among other groups. The Olmecs, a culture existing some three millennia ago, were possibly the first users of chocolate. The Olmecs are best known today for the huge stone 'fat heads' that they left dotted around Tabasco. In some forms, the Aztecs used chocolate to represent blood, a symbol made visually potent by the addi-

tion of achiote, a red material obtained from the seeds of the plant *Bixa orellana*. Cacao seeds had sufficient value and sufficient durability that they were uniformly used as currency among Mesoamerican cultures.

The second half of the book is concerned with the history of chocolate in the west (including post-conquest America). A roll call of famous names appears, including Saint-Simon, Voltaire, Francesco Redi (introducing jasmine-flavoured chocolate to Italy) and Samuel Pepys, each scampering across the page in his few sentences of chocolate-associated fame.

Sophie Coe, who died after writing preliminary versions of only the first two chapters, appears to have taken Samuel Johnson's dictum to heart and to have turned over half the libraries in Europe in preparing for the book. She earned the right to criticize others for substituting speculation for research. The book was started by her and finished by her husband, perhaps accounting for the stylistic difficulties and scattered discussions. The sensitivity shown to Mesoamerican cultures contrasts strangely with repeated derogatory comments on western concepts of the conquest period.

For example, western medicine of the era is described as "a worthless and often destructive constellation of medical theories". The theory of humors is described as being Galenic nonsense and useless. This depends on what one means by "useless". In terms of therapeutics, it has obviously been superseded. But this theory helped to organize and hold together the European world, and one could argue that our secretions, hormones and neurotransmitters are

conceptually not so different from humors. Besides, it ill befits an age that spends its time, money and intellectual resources on herbs, elixirs and 'natural' cures to make fun of the invalid (as distinct from irrational) beliefs of earlier generations. There is also a habit of historical comparisons, such as Cromwellian England being compared with Iran under the Ayatollah, Brazil under the Jesuits with Pol Pot's Cambodia, and so on. They have all the value of their type. The style tends to the breathless, full of those twin banes of English, adjectives and exclamations.

One will search the book in vain for meaningful discussion of the technology or enzymology of the complex changes in the so-called 'fermentation' of the cacao bean that lead to production of the characteristic chocolate flavour. Also

absent is a proper discussion of the pharmacology of chocolate. Why does it appear to have so many of the characteristics of an addictive substance? Is it not the theobromine, caffeine or sugar content that leads the chocoholic to dip and dip again until the box is empty. Is it that the fat-to-sugar ratio leads to an optimum 'mouth feel', with its hedonic associations? Does the breakdown of protein during the fermentation process lead to production of exorphins, or peptides having opioid activity, similar to the casomorphine produced in the digestion of milk?

Even the discussion of natural history is poorly focused, and the reader comes away confused as to whether the various types of criollo and forastero are different species, subspecies, cultivars or merely minor and botanically insignificant variations within the same taxon. The geographic origin of *Theobroma* is not clearly discussed. The authors appear to take issue with the conventional view that *T. cacao* is indigenous to South America. They claim a Mesoamerican origin but do not give a clear rationale for thinking this. Indeed, they point out that much of central America is climatically unsuited for *T. cacao* cultivation.

An attempt is made to rationalize the etymology. The Nahuatl (Aztec) word for the chocolate drink was *cacahuatl*, or 'cacao water'. The Mayan term was *chocol haa*, or 'hot water'. It is surmised that the Spanish compounded the two terms, taking the Mayan *chocol* and the Nahuatl *atl* to make *chocolatl*. This seems reasonable, particularly given the associations of 'caca' in Spanish, and the appearance of the drink.

Taken on its own terms, the book is an enjoyable read and cuts through some of the confusions and semi-fictions surrounding the history of this fascinating substance, more than a food but less than a drug. The taint of sin, sloth and the south are still associated with chocolate. Similarly, Protestantism, hard work and the north are associated with coffee. Why is this? Although consumed in the west mostly in the form of solid chocolate or the drink known in England as cocoa, chocolate is capable of being combined with a variety of other substances for an enhanced appeal. My favorite is chocolate *molé*, a sauce combining bitter chocolate with chilli. This is an incomparable pairing, in which the characteristics of the one ingredient complement those of the other to yield a smooth mouth sensation in which the bite of the chilli is mellowed to an indescribable richness. The *molé* is poured onto chicken or a suitable alternative. Recipe sent on request. □

Ryan J. Huxtable is in the Department of Pharmacology, University of Arizona, Tucson, Arizona 85724, USA.

Botanical cultures

David Pimentel

Plants, People, and Culture: The Science of Ethnobotany. By Michael J. Balick and Paul Alan Cox. *W. H. Freeman/Scientific American Library*: 1996. Pp. 228. \$32.95, £19.95.

Making Nature, Shaping Culture: Plant Biodiversity in Global Context. By Lawrence Busch, William B. Lacy, Jeffrey Burkhardt, Douglas Hemken, Jubel Moraga-Rojel, Timothy Koponen and José de Souza Silva. *University of Nebraska Press*: 1996. Pp. 261. \$52.50, £40.

Medicinal Resources of the Tropical Forest: Biodiversity and Its Importance to Human Health. Edited by Michael J. Balick, Elaine Elisabetsky and Sarah A. Laird. *Columbia University Press*: 1996. Pp. 440. \$75 (hbk); \$35 (pbk).

PLANTS are essential to the survival of human societies and affect nearly every aspect of our lives. As hunter-gatherers, prehistoric humans collected plants for food and fibre, a dependence that eventually led to the development of agriculture.

of the human economy. Trees provide billions of people with lumber for housing, furniture and other components of shelter. Wood fuel was the first energy source managed by humans and continues to be widely used, especially in developing countries. Paper, made not only from trees but also from many other plants, is fundamental to educational and communication systems. And plants supply cotton and various other fibres for weaving into fabrics for clothing.

Indeed, it is fair to say that plants have influenced the very trajectory of civilization. The search for new routes to the spice islands, for example, encouraged Columbus to seek a short passage there, resulting in his discovery of the West Indian Islands and North America. Sailors from Spain, England, the Netherlands, France and Portugal also ventured to distant regions in pursuit of spices and other valuable plant products. The economic value of some spices in the sixteenth century was enormous. For instance, the Dutch would purchase the equivalent of a dollar's worth of nutmeg in the Banda Islands west of New Guinea and resell the spice in Amsterdam for \$30,000.

In *Plants, People, and Culture*, Michael

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Women in the Yunnan province of China preparing hemp thread.

Although some 20,000 of the 350,000 known plant species are now used as human food, rice, wheat and corn provide 60 per cent of the total. Corn, or maize, cultivated in Mexico as early as 3000 BC, had an enormous influence on the development of cultures in Latin America. This valuable food and feed crop eventually spread throughout the world, radically changing every human culture that adopted it.

All vegetation has the unique ability to produce biomass from carbon dioxide, water and soil nutrients, as well as solar energy. Although used by humans mainly as food, plants are involved in all aspects

Balick and Paul Cox explain the versatility of plants in the human ecosystem. Hemp (*Cannabis sativa*), long a source of fibre for rope, cloth and paper, was once widely grown in the United States with government support. Its production there is now illegal because it is a source of the psychoactive drug cannabis, even though THC (tetrahydrocannabinol), a compound also found in hemp, is extremely valuable in the treatment of eye glaucoma as well as nausea and other side-effects of cancer chemotherapy.

From ancient times, plants have been a source of medicines and drugs. The authors report that nearly one out of

every four pharmaceutical drugs in use today were discovered from studying plants used by indigenous peoples for healing. The current value of plant-based drugs is estimated to be more than \$200 billion a year worldwide. As scientists explore plants and the lifestyles and diets of indigenous peoples in more and more detail, there will be many exciting prospects for discoveries of new and even better medicines, especially with the assistance of biotechnology.

Plants, People, and Culture is an exciting book, clearly written and well illustrated. Those interested in biology, geography, history, culture, food, drugs and other topics relating to plants and their interaction with humankind will find it fascinating. It impresses on readers the value of plants in their lives and cultures and the critical need to conserve plant biodiversity for future generations.

The other two volumes under review also deal with plant biodiversity and society, but are more specialist and differ from each other in their focus on the links between plant diversity and human use and culture. In *Making Nature, Shaping Culture*, the authors look at the way in which farmers over the past 10,000 years have domesticated and exploited numerous plant varieties for food, forage and fibre; rightly pointing out that scientists have 'made' nature through genetic manipulation and breeding, they then leap to the surprising conclusion that this "nature is not natural".

Medicinal Resources of the Tropical Forest focuses on ethnomedicine, ethnobotany, pharmacognosy, drug discovery and practical aspects of the chemistry of natural products. The relevance of plant-based pharmaceuticals is here confirmed by the fact that more than 75 per cent of the world's population depends on them.

The contributors to both books reiterate the importance of the conservation of biodiversity in the sustainability of food production and the search for new medicines, and to this end outline long-term strategies for practical management of plant resources. □

David Pimentel is at the College of Agriculture and Life Sciences, Cornell University, Ithaca, New York 14853-0901, USA.

■ Two other ethnobiology books have just been published. *Ethnobotany: Principles and Applications* by C. M. Cotton contains examples from throughout the world and covers a wide range of source materials. It is an introductory textbook that describes the history of the interaction between plants and people and the concepts, methods and future directions of this rapidly growing area of plant biology. Wiley, 424 pages £50 (hbk), £24.95 (pbk). *Medical Ethnobiology of the Highland Maya of Chiapas, Mexico: The Gastrointestinal Diseases* by Elois Ann Berlin and Brent Berlin draws on data on the medical practices of the Tzeltal and Tzotzil peoples. Princeton University Press, 557 pages, \$79.50, \$50.

Genetic half-truths

William H. McNeill

In the Blood: God, Genes and Destiny. By Steve Jones. HarperCollins: 1996. Pp. 302. £20.

FRICTION between theology and biology has persisted in intellectual circles ever since Darwin's time. Steve Jones, professor of genetics at University College London, therefore shows unusual bravado in taking on God, genes and destiny. He explains in the preface: "Although my own interest in matters of religion is as amateur as that of most of my colleagues, it seems to me worth exploring the alternative accounts of existence if for no other reason than to point to their parallels. Because the questions posed by theology and by genetics are so similar, *In the Blood* is, very loosely, structured around biblical themes: Adam and Eve, Original Sin, the Expulsion of the People of Israel, Resurrection, Armageddon, the Last Judgment and much more". The result is an unusual set of chapter headings for a book on genetics.

The explanation for this oddity is that *In the Blood* was written to accompany a BBC television series with the same title. Interweaving genetics with the Bible is presumably a good way of attracting popular attention. But conforming to the visual rhetoric of the television screen (and the short attention span of most viewers) produces expository prose that hurries from topic to topic, mimicking a butterfly fluttering from flower to flower in search of nectar. The crowded assemblage of oddments of information that results is always interesting, and only occasionally disorienting to a reader as ignorant as I am of recent discoveries in genetics and of some of the sectarian byways that Jones touches upon.

On the whole, I would have preferred a more straightforward scientific account of what is now known and what remains unknown, for, as Jones points out, since the discovery of the structure of DNA in 1953, geneticists have "done a good job in educating society about what their science can do" while remaining "coy about what their subject has not achieved". Presumably, his three earlier books did just this: but even if, as Jones says, "a word is worth a thousand pictures when they come as frames of film", this book uses words to construct parallels between biblical theology and biology that are never exact — and so not really parallel at all.

The passages dealing with aspects of what is now known about human genetics are instructive and fascinating. Jones's remarks about melanin and its diverse roles in the human brain (as well as skin) were new to me, as was his account of a

current campaign, based mainly in New York, to revalue the blackness that melanin gives to African skins by attributing truly extraordinary powers to this compound. But his conclusions about the genetics of race are entirely familiar: "Genetics means that now, at last, perceptions of human quality need not depend on what is on the surface. For the first time it is possible to form an impartial view of just how different races might be. The answer is clear. The biological differences among them are small and the evidence for the mental superiority of one or the other so flimsy, confused and full of intellectual dishonesty as to be scarcely worth considering".

No surprise in that. By contrast, many lay readers will find what Jones has to say in his final chapter about the genetics of ageing and rejuvenation through sexual reproduction both surprising and enlightening. In his words: "Genetics casts new light on the alliance of sex, age and death. It shows how all derive from errors, mutations, in copying the genetic message. They cause individuals to decay but genes to be preserved. Each generation, sex redeems the germ plasm and is the stuff of the evolutionary change that ensures its long-term survival. More and more it seems that this sexual renewal is paid for by somatic degeneration... that beset[s] everyone who reaches old age".

Jones's closing pages highlight the implausibility of human (or any other kind of) life. How amazing it is that "six feet of DNA" coiled into a single cell can summon an adult human being into existence whose cells, if he is male, then proceed to make "tens of thousands or miles of DNA an hour". He continues the argument by pointing out that "[t]he main task of sex is to undo the damage caused by mutation", and after describing the complex chemical mechanisms involved in correcting the genetic code he concludes his book as follows: "St Paul himself described the conflict between the fate of the genetic message in the body and that in the cells which transmit it to the next generation in terms familiar to a biologist: 'For this corruptible must put on incorruption and this mortal must put on immortality.' Biology shows how right he was: that, although the life of those who bear them is transient, the world of the genes will live on for ever".

This quotation epitomizes the intellectual corruption I find at the core of *In the Blood*. However striking the comparison Jones is making, it is simply not true that this verse from I Corinthians 15:53 "describes the fate of the genetic message in the body". Still less is it true that St Paul's words use "terms familiar to a biologist". The apostle was writing about Judgment Day when "the trumpet shall sound, and the dead shall be raised incorruptible, and we shall be changed", as the

immediately preceding verse explains. Equating St Paul's vision of things to come with contemporary geneticists' ideas about the continuity and change of flesh and blood across generations is clever but meretricious.

I assume the author to be both expert in and accurate about genes, yet this final *jeu d'esprit* is also biologically absurd. Things — and especially living things — do not endure forever. Even the hardest and most ingenious genes can scarcely outlast the Earth, and astronomers now agree that our Earth and Sun have a limited lifespan. I was also bothered by other instances of exaggeration. For example: "The Mormons' Ancestral File proves that by going back far enough all family trees sooner or later coalesce, meeting in an individual who links them". This cannot be true. What about New Guinea tribesmen who have been isolated from Mormon ancestors for at least 40,000 years? Assuredly, genetic resemblances attest to common ancestors for them and

all the rest of us: but the Mormon files do not prove it. All they can possibly show is that across the past two or three millennia, geographical contacts among human populations resulted in intermarriage (or at least interbreeding), thereby connecting previously distinct lineages. But even today among a few isolated populations, genetic links with the rest of humankind are so ancient as to escape all written record.

But such slips — and a few historical errors as well — do not really matter. What does matter is the rich array of historical and religious oddments that Jones has gathered together to illustrate aspects of genetics as currently understood. The combination makes for a surprising, instructive, confusing and occasionally irritating book. □

William H. McNeill, emeritus professor of history at the University of Chicago, is at 36 School House Road, Colebrook, Connecticut 06021, USA.

progression of chords in the French horns as the cold comes, ice forms, thickens and begins to flow... sometimes this melody is fierce and improvisatory, as the ice cracks and creaks...". The irritation comes not from these musical asides, but from the relentless, pointless, mystifying jokiness of the book, inadequately represented by this tiny sample: "Based on age dating, the ages of most diamonds are either 3.3 billion years old (before life), 1,150 million years old (before the Volvo) or 950 million years old (before CD players)". In his acknowledgements, Morton candidly states that his editor had put "great slashing blue lines through most of what she referred to as my cornball humor". Given what survives, one can only boggle at the scale of what was omitted.

Most readers welcome touches of humor in technical texts. Apart from lightening conceptually demanding passages, they also permit the author's personality to shine through. Morton's mistake is of allowing his personality to obtrude. If he had spent less effort trying to be funny, and a little extra on weeding out the profusion of factual errors, his book would have been more useful. As it stands, it ought to carry a health warning for readers with hypertension.

Little of MacDougall's personality shows through in his *Short History of Planet Earth*, billed on the cover as "the ideal concise introduction to earth and life sciences". In his ambitious account, MacDougall attempts to review both the evolution of the solid Earth and the life forms on it. Apart from a sprinkling of jarring clichés — "these structures are mute testament to the tremendous compressive forces that occur when plates collide" — his account is instructive and readable. By adopting a narrative form, he presumably intends his book to be read by nonspecialists. Although individual paragraphs and pages are perfectly digestible, many lay readers may lack the stamina to get to the end, given the inevitably large volume of factual detail involved. To be successful, a narrative account needs to offer changing tempos and varying vistas. MacDougall's steadily paced, slightly plodding account can be rather soporific. Readers with a geological background may enjoy it, in the same way that one enjoys hearing a favourite Mozart opera for the umpteenth time.

So what is the secret to producing a successful introductory Earth science text? Lyell had it right: complete authority over the subject (he more or less invented it) and a simple incisive writing style. Both Morton and MacDougall would do well to go back and seek that end of the spectrum. □

P. W. Francis is in the Department of Earth Sciences, Open University, Milton Keynes MK7 6AA, UK.

A discordant duo

P. W. Francis

Music of the Earth: Volcanoes, Earthquakes, and Other Geological Wonders. By Ron L. Morton. Plenum: 1996. Pp. 312. \$28.95.

A Short History of Planet Earth: Mountains, Mammals, Fire and Ice. By J. D. MacDougall. Wiley: 1996. Pp. 266. \$24.95, £19.99.

I HAVE found the end of the rainbow — or at least one end of a spectrum. According to fairy tales, I should have found a crock of gold there. What I actually found was a lot less appealing; more like a pile of irritating small-denomination foreign coins. The spectrum whose vexatious end I located was that of introductory Earth science texts, which span a vast range of scholarship and literacy. Ever since Lyell's classic three-volume *Principles of Geology*, which was first published between 1830 and 1833 and went through numerous subsequent editions, there has been a steady demand for elementary texts. More appear every year. Their basic ingredients are fairly standard: the origin and internal structure of the Earth, rocks and minerals, the geological timescale, chapters about plate tectonics, volcanoes and earthquakes, the origin and evolution of life, dinosaurs, mass extinctions and so on. There are rainbow-coloured shelves full of such books, mostly designed for first-year US college students.

Any author wishing to break in to this potentially lucrative field must have something special to offer. One way is to produce a yet more glossy and lavishly

illustrated text than any of its predecessors. Another is to offer a personal or idiosyncratic view of the subject. Neither of the books reviewed here has favoured the glossy route — in fact both are so sparsely illustrated as to be rather drab. MacDougall's book has a few line diagrams, and no photographs at all. Both authors seem to have elected to try the individual approach, MacDougall through a synoptic account of Earth history, Morton through a far more eccentric survey of "Volcanoes, Earthquakes, and Other Geological Wonders".

It is clear even from the dustjacket of Morton's book why it lies at the vexatious end of the spectrum. The blurb claims that: "In a stunning blend of poetry, music and science this lyrical new work evokes the wonders of our living earth. As Pythagoras marvelled over the 'music of the spheres' geologist and writer Ron Morton gives us a whole new appreciation of the awe-inspiring forces that make and shape our planet". What Morton actually gives is a clear demonstration of the dangers of going over the top. Nothing fails like excess, to coin a jokey aphorism of the sort that Morton might appreciate.

Although more than a little strained, the attempt to portray geological processes in musical terms is innocuous enough. It amounts to little more than a 'prelude' to each chapter, in which Morton uses musical metaphors to recreate the appropriate atmosphere. In places these are actually quite evocative, as in the prelude to "Winter's Breath", a chapter on the Ice Age: "Hear the slow harmonic

Major-element variability in the Hawaiian mantle plume

Erik H. Hauri

Department of Terrestrial Magnetism, Carnegie Institution of Washington, 5241 Broad Branch Road NW, Washington DC 20015, USA

Isotope ratios and concentrations of silica, titanium, aluminium, calcium, sodium and iron are correlated in shield-stage basaltic lavas erupted over the past three million years from eight Hawaiian volcanoes. These correlations indicate the presence of a component beneath Hawaii with a bulk composition which is distinct from typical mantle peridotite. This material is most probably recycled oceanic crust distributed within the Hawaiian plume as segregations of quartz-bearing garnet pyroxenite or eclogite, which yield silica-rich (dacitic) magmas on partial melting.

The existence of isotopic heterogeneity in the Earth's mantle is beyond dispute. Even in the part of the mantle that is subject to convective motions and mixing on geological timescales, isotopic variability in the elements helium, strontium, neodymium, lead and osmium is clearly observed in lavas erupted at ocean ridges and hotspots¹⁻⁴. Isotopic heterogeneity in oceanic basalts requires the presence of distinct mantle reservoirs with different parent/daughter ratios ((U + Th)/He, Rb/Sr, Sm/Nd, (U + Th)/Pb and Re/Os) which have independently survived in the convecting mantle for periods of time as long as several billion years (refs 1-5). Yet it remains to be determined how this isotopic variability relates to mantle heterogeneity in trace elements (abundances <1 wt%) and major elements (abundances >1 wt%). Correlations between isotopes and some trace elements in mantle-derived lavas indicate trace-element variability in some regions of the Earth's interior⁶⁻⁹, but it is not yet certain whether this isotopic heterogeneity is also indicative of a similar variability in the major-element compositions of mantle sources.

The Earth's upper mantle may be the best example of the uncertain relationship between isotopes and major elements. One of the most significant accomplishments of mantle geochemistry has been the demonstration that extraction of the continental crust from the Earth's interior has left a complementary 'depleted' upper mantle^{1,10}. Mid-ocean-ridge basalts (MORBs) derived from this depleted mantle have depleted trace-element and isotopic characteristics, but it has not yet been possible to determine if this part of the mantle is depleted in any of the major elements (Si, Al, Fe, Ca). Indeed, mantle peridotites with depleted isotope ratios similar to MORB are usually fertile in their major-element composition, and do not differ substantially from the estimated major-element composition of undepleted mantle¹¹⁻¹⁶. Conversely, peridotites that are strongly depleted in the major elements Ca, Al and Fe (harzburgites) often show complicated signatures of enrichment in trace elements and some isotope ratios^{12,17-19}. As a result of this decoupling of major elements, trace elements, and isotope ratios in peridotites, it has been difficult to determine whether isotopic variations in mantle-derived lavas are associated with large differences in the major-element composition of their mantle sources.

Here I show that shield-stage lavas from Hawaiian volcanoes display correlated variations in isotope ratios and major elements which cannot be explained either by shallow-level processes such as alteration and crustal contamination, or by deeper processes such as mantle-magma reaction or variable partial melting of a homogeneous source. Although partial melting may result in some major-element variability, melting effects are superimposed on a large variability in the major-element composition of the

solid mantle beneath Hawaii, as indicated by correlations with isotope ratios which are not affected by the melting process.

Isotope/major-element correlations

Major-element and isotope data for the Hawaiian volcanoes Loihi, Kilauea, Mauna Loa, Mauna Kea, Kohala, Kahoolawe, Haleakala and Koolau come from literature sources²⁰⁻⁴⁷; only lavas with major-element and isotope analyses on the same sample are used. This study is restricted to analyses of tholeiitic and alkalic Hawaiian basalts with >6.5 wt% MgO which erupted during the pre-shield and shield stages of volcano evolution; lavas erupted during these two stages typically make up >95% of the volumes of individual Hawaiian volcanoes. We consider no basalts from the occurrences of intercalated tholeiites and alkali basalts which are products of the post-shield stages of volcanism, as these lavas sometimes show evidence of extensive low-pressure differentiation and assimilation^{48,49}. Other volcanoes from the islands of Hawaii, Maui, Molokai and Oahu are not considered here owing to lack of published data or poor exposure of shield lavas. A majority of the basalts (74%) have $K_2O/P_2O_5 > 1$, indicating minimal post-eruptive alteration (which typically results in loss of K_2O). Most of the lavas with $K_2O/P_2O_5 < 1$ come from the three oldest volcanoes examined in this study, Kohala, Kahoolawe and Koolau.

Isotope ratios of Sr, Nd, Pb and Os are well correlated with measured abundances of SiO_2 , TiO_2 , Al_2O_3 , FeO and CaO in Hawaiian lavas. However, some of the measured major-element variability is due to removal or accumulation of olivine under low-pressure conditions. To correct for the effects of olivine fractionation, the Hawaiian major-element data have been adjusted to a constant $Mg/(Mg + Fe^{2+})$ ratio ($Mg\#$) by adding or subtracting olivine in 0.1% increments, assuming 10% of the total iron as Fe^{3+} and using an olivine-melt K_d of 0.30 (ref. 50; $K_d = (Fe^{2+}/Mg)_{olivine}/(Fe^{2+}/Mg)_{melt}$). For analyses with $MgO < 18\%$, the olivine composition used was that in equilibrium with the bulk rock; for analyses with $MgO > 18\%$, an olivine with a forsterite content of 87% was used. After applying this correction, the correlations between isotope ratios and major-element abundances are little changed, despite a significant reduction in the variance of the major-element data.

Average compositions of individual suites of Hawaiian lavas are given in Table 1. Examples of some of the trends of isotope ratios plotted versus major elements are shown in Fig. 1. The correlations are significant at the >99% confidence level for all the possible correlations between the fractionation-corrected abundances of SiO_2 , TiO_2 , Al_2O_3 , FeO , Na_2O , CaO and CaO/Al_2O_3 and the isotope ratios $^{143}Nd/^{144}Nd$, $^{87}Sr/^{86}Sr$, $^{206}Pb/^{204}Pb$, $^3He/^4He$ and

$^{187}\text{Os}/^{188}\text{Os}$. The only exceptions are $^3\text{He}/^4\text{He}$ versus TiO_2 (95% confidence), $^3\text{He}/^4\text{He}$ versus FeO (75% confidence), and $^3\text{He}/^4\text{He}$ versus Al_2O_3 (no correlation). The correlations are independent of $\text{K}_2\text{O}/\text{P}_2\text{O}_5$ ratio, demonstrating that these relationships are primary features of the lavas unrelated to post-eruptive alteration in the tropical environment.

The data in Fig. 1 are separated into two groups based on the location of the individual volcanoes along two structural lineaments in the Hawaiian Islands⁵¹, termed the Loa trend (including Loihi, Mauna Loa, Kahoolawe and Koolau) and the Kea trend (including Kilauea, Mauna Kea, Kohala and Haleakala). The correlations between isotopes and major elements are best among the Loa trend volcanoes. Addition of the Kea trend volcanoes increases the scatter in the correlations; the compositions of Kea trend volcanoes are shifted away from the Loa trend correlation towards MORB (Fig. 1). The extremes of the correlations are defined by data from Koolau volcano (low $^{143}\text{Nd}/^{144}\text{Nd}$, high SiO_2) and Loihi seamount (high $^{143}\text{Nd}/^{144}\text{Nd}$, low SiO_2), both Loa trend volcanoes. Although alkalic and tholeiitic basalts are both present at Loihi, the two groups are distinctive only in their fractionation-corrected SiO_2 contents. Alkali basalts at Loihi are thought to be derived from smaller degrees of partial melting of the same source that yields Loihi tholeiites²⁴.

Major elements: crustal or mantle control?

As only lavas with $>6.5\%$ MgO have been considered, variations in the raw major-element data are dominated by fractionation and accumulation of olivine $\pm$ Cr-spinel. After correcting for olivine fractionation, large ranges in major-element composition remain, and preserve correlations with isotope ratios. Two lines of evidence argue against high-level assimilation as the origin of the isotope/major-element correlations. First, the isotopes show no correlation with MgO in these lavas, even within individual volcanoes. Second, possible assimilants beneath Hawaii such as seawater-altered mid-ocean-ridge basalt or oceanic sediments in the vicinity of Hawaii are quite unlike any Hawaiian basalts or possible endmembers to the basalt data. In addition, Os isotope data for a subset of these samples^{30,52,53} (J. Lassiter and E.H.H., unpublished data) are also correlated with the major elements (Fig. 1), but none of the samples shows signs of the highly elevated and variable Os isotope ratios characteristic of crustal contamination, such as are observed in low-MgO basalts from Haleakala⁴⁹.

These arguments all point to a mantle origin for the correlated isotope and major-element variations. Magma–mantle reaction can change the major-element concentrations of migrating basaltic magmas; in particular, pyroxene assimilation can increase the magma SiO_2 content with increasing reaction⁵⁴. But with increasing SiO_2 , Hawaiian basalts have progressively higher $^{87}\text{Sr}/^{86}\text{Sr}$ and lower $^{143}\text{Nd}/^{144}\text{Nd}$ ratios, moving away from

the isotopic composition of the depleted mantle and lithospheric xenoliths from Hawaii^{55,56}. Elevated osmium isotope ratios in Hawaiian lavas also rule out magma–mantle reaction within the oceanic lithosphere⁵³.

Variations in the partial melting process probably contribute to some of the major-element variability in Hawaiian shield lavas, such as co-existing alkalic and tholeiitic lavas at Loihi²⁴. However, the SiO_2 abundances of most Hawaiian tholeiites are too high to have been generated by peridotite melting at sub-lithospheric depths (Fig. 2). The SiO_2 content of Koolau lavas would appear to suggest a depth of melting of 30–45 km, but this depth is 15–30 km shallower than the base of the sub-Hawaiian lithosphere⁵⁷, and the elevated Os isotope ratios in Hawaiian lavas rule out a contribution from the lithospheric mantle⁵³. The SiO_2 -enriched Koolau component is not an isolated occurrence; it also occurs (though in a less extreme form) at Kahoolawe and Mauna Loa volcanoes, where this variation is accompanied by correlated shifts in the isotope ratios (Fig. 1). This phenomenon would not be expected if the major-element variations were completely controlled by

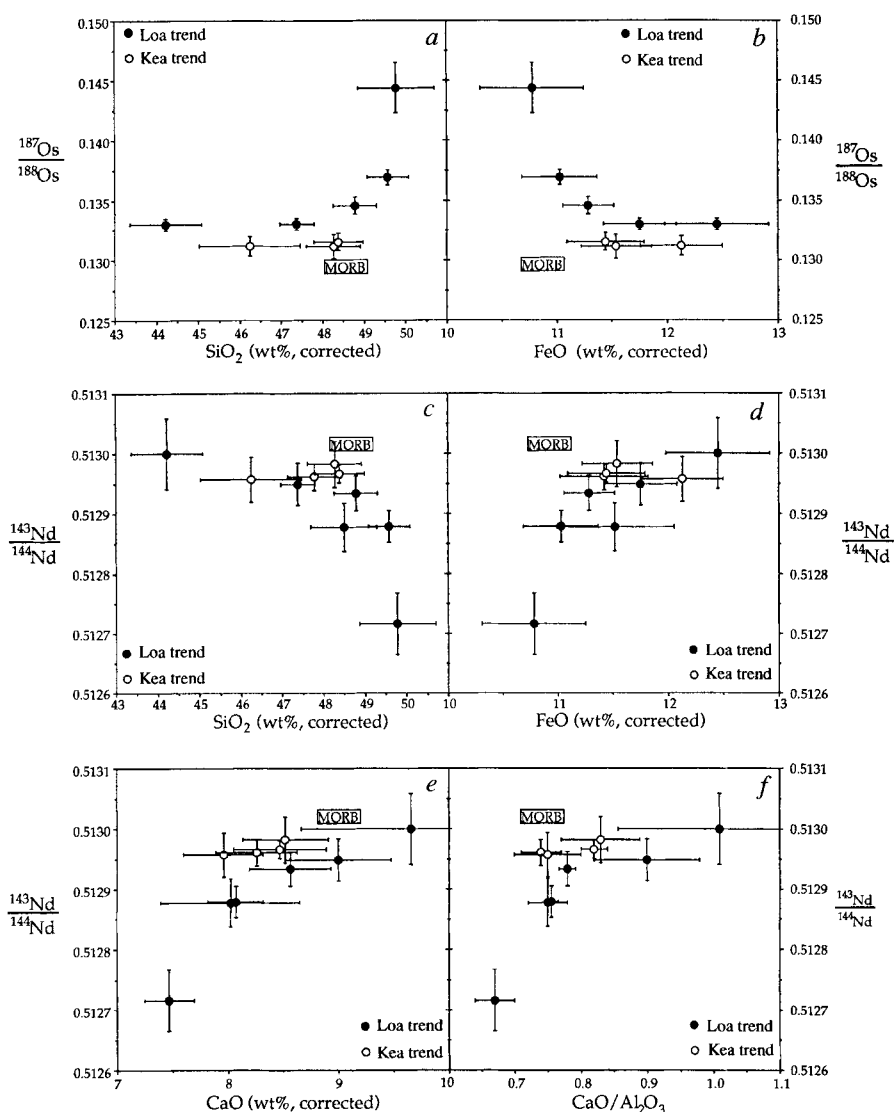


FIG. 1 Correlations of isotope ratios and major elements in Hawaiian shield lavas with $>6.5\text{ wt}\%$ MgO. Data points represent average compositions of lavas or groups of lavas from individual Hawaiian volcanoes (Table 1); error bars represent one standard deviation for each group of lavas. Major-element abundances have been corrected for olivine fractionation to $\text{Mg}/(\text{Mg} + \text{Fe}^{2+}) = 0.76$ (see text). Compositional averages are distinguished by the location of individual volcanoes over two structural lineaments termed the 'Loa trend' (solid symbols) and 'Kea trend' (open symbols)⁵¹. The box for MORB is estimated from Hofmann¹⁰, calculated to $\text{Mg}/(\text{Mg} + \text{Fe}^{2+}) = 0.76$.

partial melting of a compositionally uniform mantle source. The major-element systematics of MORB have been successfully explained by variations in the mean pressure and temperature of melting of a compositionally uniform source similar to fertile upper-mantle lherzolite^{58–60}, but such a model provides an unsatisfactory fit to the variations in major elements and isotopes observed in Hawaiian basalts. The most straightforward explanation for the correlations in Fig. 1 is that some component in the mantle beneath Hawaii is generating melts that have high SiO₂, Al₂O₃, Na₂O, ⁸⁷Sr/⁸⁶Sr and ¹⁸⁷Os/¹⁸⁸Os, and low FeO, CaO, CaO/Al₂O₃, ¹⁴³Nd/¹⁴⁴Nd and ²⁰⁶Pb/²⁰⁴Pb compared with melts of upper-mantle peridotite.

Constraints on Hawaiian source compositions

From the correlation between fractionation-corrected iron and silica contents (Fig. 2), it appears that only the low-SiO₂ end of the correlation (Loihi) could possibly be generated by partial melting of typical mantle peridotite (Mg# 0.89), and then only at very high mean pressures (>3 GPa). This would also suggest that some Hawaiian primary magmas may be higher in MgO (>20%) and Mg# (>0.77) than shown in Fig. 2a. Loihi-type magmas could also be generated at slightly lower temperature and pressure by melting Fe-enriched lherzolite, such as the peridotite HK-66 (Mg# 0.86) studied by Hirose and Kushiro⁶¹ (Fig. 2b). This possibility has been suggested previously^{62–64}. However, the degree of iron enrichment in the Hawaiian source is limited by Fe–Mg relationships that require the generation of magmas with Mg# of at least 0.77, in equilibrium with the most FeO-poor olivines found in lavas from Loihi, Kilauea and Mauna Kea. The SiO₂-rich end of the trend (Koolau) is the most enigmatic; Koolau lavas have high SiO₂, Al₂O₃ and Na₂O, and low FeO, CaO and CaO/Al₂O₃ which cannot be generated by melting either of the upper-mantle lherzolites under any conditions (Fig. 2).

The elevated Os isotopic signatures at Mauna Loa and other Hawaiian volcanoes, coupled with the Os isotope correlation with SiO₂ and FeO, suggest that the enriched component beneath Hawaii may be explained by the presence of recycled oceanic crust; such a component has been proposed for many hot-spots⁴. It is proposed here that most Hawaiian lavas, in particular those from Koolau, contain a significant fraction of SiO₂-rich melt derived by melting of mafic material in which olivine is not a stable phase (quartz-bearing garnet pyroxenite or eclogite). The subsolidus mineralogy of MORB at pressures >2.5 GPa is clinopyroxene + garnet + quartz, and many geochemical and experimental melting studies have demonstrated that dacitic magmas are produced by partial melting of quartz eclogite at high pressures^{65–67}. Thermodynamic calculations with the MELTS modelling program⁶⁸ support the idea that anhydrous partial melt-

ing of average N-type MORB at pressures >2 GPa produces dacitic melts that are rich in SiO₂, Al₂O₃ and Na₂O, depleted in FeO and MgO, and with low CaO and CaO/Al₂O₃. Dacitic melts derived from partial melting of quartz eclogite have all the major-element features that characterize the Koolau endmember, and the isotopic composition of this endmember is consistent with a significant proportion of recycled MORB crust⁵³.

A three-component, zoned Hawaiian plume

The major-element variability in Hawaiian shield lavas is dominated by the Koolau component, which is largely restricted to Loa trend volcanoes. However the apparent curvature between the Loihi and Koolau components (Fig. 1) cannot be matched by a single mixing curve since all reasonable mixing curves on the isotope/major-element diagrams are nearly linear. This indicates that a three-component model is required to explain the data. This observation is supported by a principal-component analysis utilizing major-element and isotopic data, which indicates that three endmembers account for over 95% of the variance in the data. Following previous convention, the three identified components are termed Loihi, Koolau and Kea.

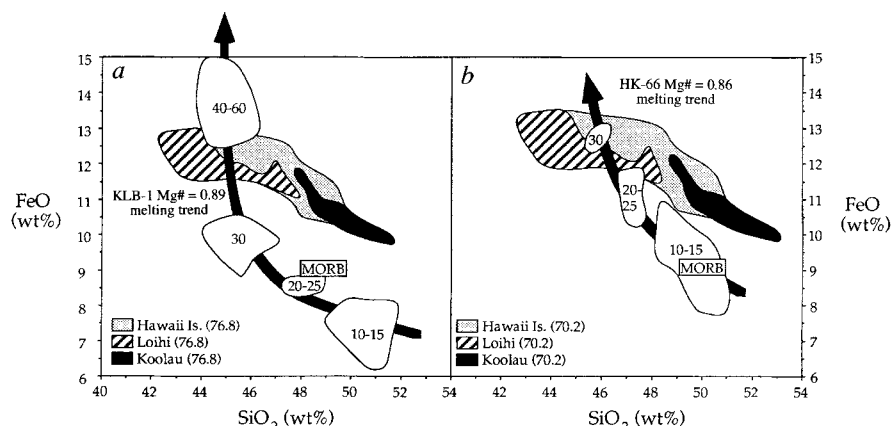
Isotopic and major-element estimates of the three endmember magma compositions (Table 2) show that the Koolau component has a very distinctive composition. The Loihi and Kea components are similar in major elements but distinct in isotopes. The Loihi and Kea melts can be reasonably derived by partial melting of mantle peridotite, but the Koolau melt component requires

TABLE 1 Average compositions of stratigraphic sections from Hawaiian volcanoes

Loa trend						
	Loihi tholeiitic	Loihi alkaline	Mauna Loa (>28 kyr)	Mauna Loa (<28 kyr)	Kahoolawe	Koolau
SiO ₂	47.53	44.59	48.77	49.79	48.54	49.98
TiO ₂	1.97	2.10	1.72	1.67	1.73	1.65
Al ₂ O ₃	10.13	9.71	11.00	10.86	10.86	11.34
FeO	11.80	12.57	11.29	11.08	11.54	10.82
MgO	17.09	18.14	16.27	16.00	16.66	15.62
MnO	0.16	0.16	0.15	0.14	0.14	0.14
CaO	9.14	9.79	8.57	8.21	8.14	7.60
K ₂ O	0.29	0.66	0.22	0.32	0.30	0.36
Na ₂ O	1.70	2.03	1.84	1.76	1.90	2.28
P ₂ O ₅	0.18	0.26	0.16	0.18	0.19	0.22
³ He/ ⁴ He (R/R _a)	27.0	26.8	16.4	9.49	ND	13.0
⁸⁷ Sr/ ⁸⁶ Sr	0.70360	0.70347	0.70370	0.70388	0.70410	0.70422
¹⁴³ Nd/ ¹⁴⁴ Nd	0.512949	0.513000	0.512934	0.512879	0.512877	0.512716
²⁰⁶ Pb/ ²⁰⁴ Pb	18.380	18.373	18.191	18.112	18.093	17.853
¹⁸⁷ Os/ ¹⁸⁸ Os	0.1330	0.1330	0.1346	0.1370	ND	0.1444
Kea trend						
	Kilauea	Mauna Kea	Kohala	Haleakala		
SiO ₂	48.45	48.43	48.04	46.46		
TiO ₂	2.01	2.01	1.65	2.06		
Al ₂ O ₃	10.47	10.29	11.34	10.85		
FeO	11.46	11.58	11.50	12.20		
MgO	16.54	16.64	16.65	17.65		
MnO	0.15	0.17	0.16	0.16		
CaO	8.59	8.55	8.42	8.07		
K ₂ O	0.34	0.31	0.32	0.39		
Na ₂ O	1.79	1.84	1.75	1.93		
P ₂ O ₅	0.19	0.18	0.16	0.22		
³ He/ ⁴ He (R/R _a)	16.7	9.0	10.0	14.3		
⁸⁷ Sr/ ⁸⁶ Sr	0.70358	0.70359	0.70369	0.70361		
¹⁴³ Nd/ ¹⁴⁴ Nd	0.512966	0.512982	0.512961	0.512957		
²⁰⁶ Pb/ ²⁰⁴ Pb	18.534	18.440	ND	18.300		
¹⁸⁷ Os/ ¹⁸⁸ Os	0.1315	0.1311	ND	0.1312		

These compositions are corrected for olivine fractionation to Mg# = 0.76 (see text). ND, no data. R/R_a, ³He/⁴He relative to the atmospheric ratio (1.39 × 10⁻⁶).

FIG. 2 FeO abundances versus SiO₂ abundances for Hawaiian lavas and experimental melts in equilibrium with mantle peridotite^{61,74–78}. Abundances for Hawaiian lavas are corrected for olivine fractionation to two different Mg#, corresponding to the average Mg/(Mg + Fe²⁺) of melts of upper-mantle peridotite (0.77) and Fe-rich peridotite (0.70). Experimental data are plotted as reported. Shaded field encloses all Hawaiian shield lavas with >6.5 wt% MgO; hatched and solid fields enclose data for Loihi and Koolau volcanoes, respectively. Open fields enclose data for partial melts (in equilibrium with residual clinopyroxene) of fertile upper-mantle spinel lherzolite (a, Mg# = 0.89)^{61,74–78} and Fe-rich Hawaiian spinel lherzolite (b, Mg# = 0.86)⁶¹; numbers in fields indicate experimental pressures in kbar. Most Hawaiian lavas are too high in SiO₂ at a given FeO to have been derived from garnet and clinopyroxene-bearing mantle peridotite.



partial melting of a mafic source. A least-squares fit of the Hawaiian data to these three melt components indicates that Koolau lavas contain on average about 14% dacitic melt (Fig. 3), and a maximum of about 20% for the most SiO₂-rich Koolau lava. On decompression, the quartz eclogite component will yield more melt than the surrounding peridotite; melt productivity calculations of a mixed pyroxenite-peridotite source⁶⁹ suggest that the mass proportion of quartz eclogite component in the Hawaiian plume varies from 0% (Haleakala) to at least 5% (Koolau).

The chemical differences between the spatially distinct Loa and Kea trends provide an observation which constrains the spatial relationships of the three components identified above. Figure 4 illustrates a schematic model of the Hawaiian plume beneath the Pacific lithosphere which takes into account the isotope/major-element systematics, and is consistent with the compositional differences between coeval Hawaiian volcanoes⁷⁰. The small amount of Koolau component in Kea trend volcanoes, and the upper-mantle component in Kea trend lavas identified from isotopes, suggest that the quartz eclogite component is probably restricted to the centre of a concentrically zoned Hawaiian plume located beneath the Loa trend volcanoes. The vertical distribution of eclogite in the plume may be uneven, as individual Loa trend volcanoes show variable amounts of this component (Fig. 3). The upper-mantle Kea component is identified as passively upwelling

asthenosphere surrounding the Hawaiian plume and contributes most significantly to Kea trend volcanoes located over the periphery of the Hawaiian plume.

Implications for mantle plumes

Based on the variability in both isotopes and major elements within the Koolau lava suite, the Koolau component is envisioned as lithologically distinct segregations of quartz eclogite within a peridotite matrix. This is in contrast to the compositional homogeneity of lavas from the Pacific islands of Tubuai and Mangaia, which are thought to contain a substantial mafic component which has been lithologically homogenized⁴. The difference in average SiO₂ contents in primitive lavas from Tubuai–Mangaia (43%) and Koolau (50%) may reflect differences in the SiO₂ content of their eclogitic sources. The isotopic compositions of these groups indicate that the former may have experienced sediment removal and/or extraction of SiO₂-rich melt during subduction, whereas the latter appears to have retained a sedimentary component⁵³. Contrary to the recent results of Bennett *et al.*⁴⁷, our major-element and isotopic analysis provides strong support for

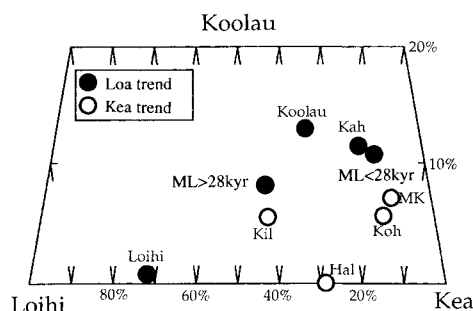


FIG. 3 Ternary diagram ($\times 3$ vertical exaggeration) showing the results of a least-squares fit of the average Hawaiian compositions to the Loihi, Kea and Koolau components; these components were estimated from data correlations identified from a principal-component analysis utilizing major-element abundances and isotope ratios of He, Sr, Nd, Pb and Os in 157 shield-stage Hawaiian lavas. The Koolau component is a dacitic melt derived from partial melting of quartz eclogite. Abbreviations for volcano names as follows: ML > 28 kyr, Mauna Loa > 28,000 years old; Kah, Kahoolawe; Koh, Kohala; Kil, Kilauea; MK, Mauna Kea; Hal, Haleakala.

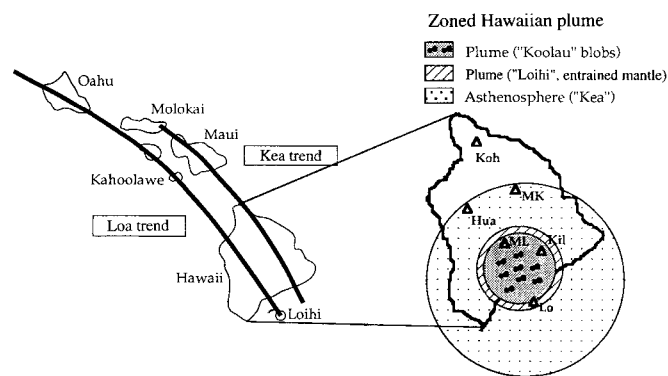


FIG. 4 Schematic model of a zoned mantle plume beneath Hawaii; Loa trend and Kea trend are structural lineaments of volcanic shields identified previously⁵¹. The plume is zoned owing to the effects of thermal entrainment⁷⁹. The centre of the zoned plume contains quartz eclogite blobs (Koolau component) in a peridotite matrix, while the outer zones consist of heated and entrained lower mantle (Loihi component), surrounded by passively upwelling (not heated) upper-mantle asthenosphere (Kea component). The passage of Loa Trend volcanoes over the plume centre causes them to have larger amounts of the Koolau component, whereas passage of Kea trend volcanoes over the periphery of the plume results in a larger proportion of the upper-mantle Kea component in these volcanoes⁵³. Abbreviations for volcano names: Hua, Hualalai; others as in Fig. 3.

TABLE 2 Estimated compositions of endmember melt components in Hawaiian lavas

	'Loihi'	'Kea'	'Koolau'
SiO ₂	47.22	47.00	64.00
TiO ₂	2.20	1.90	0.25
Al ₂ O ₃	10.00	10.00	16.35
FeO*	12.00	11.90	6.00
MgO	17.50	18.40	6.00
CaO	9.00	9.27	1.50
Na ₂ O	1.75	1.40	4.50
K ₂ O	0.33	0.13	1.40
³ He/ ⁴ He (R/R _a)	32	8.0	0.1
⁸⁷ Sr/ ⁸⁶ Sr	0.7037	0.7030	0.7080
¹⁴³ Nd/ ¹⁴⁴ Nd	0.5129	0.5132	0.5113
²⁰⁶ Pb/ ²⁰⁴ Pb	18.4	18.7	15.2
¹⁸⁷ Os/ ¹⁸⁸ Os	0.133	0.128	0.21

For simplicity, identical concentrations of He, Sr, Nd, Pb and Os have been assumed for all endmembers. FeO*, total iron as Fe²⁺.

the presence of a crustal component in the Hawaiian mantle plume.

Nearly all previous geophysical and geochemical models for major-element variability in the Earth's mantle have called on some form of crystal segregation and subsequent layering during

cooling of a global magma ocean early in Earth's history (see, for example, ref. 71 and references therein). Although it seems difficult to envision an initial stage in which the Earth was not largely molten⁷², results of high-pressure partitioning experiments⁷³ indicate that Hawaiian lavas have neither trace-element nor isotopic compositions which would be expected of the crystal products of magma ocean differentiation. Instead, recycling of oceanic crust would appear to be the most important mechanism for producing major-element variability in the Earth's interior.

The proposed origin of the Koolau component as recycled oceanic crust, and its preservation as lithologically distinct eclogite, might suggest that such a component could be detectable by seismic methods. However, at 5% by mass, a single eclogite cylinder would have a diameter of no more than 18 km in an 80-km-diameter plume, and would be more likely to be distributed within the plume and thus be even smaller in scale. Geochemistry is probably the most sensitive means of detecting major-element variability in the mantle, especially where clear major-element/isotope correlations can be identified. In addition to the widely held view of major-element control by partial melting^{58–61,70}, the analysis presented here indicates that the major-element systematics of some mantle-derived lavas may be a reflection of major-element heterogeneity in the mantle. The correlated isotope and major-element compositions of melt derived from a recycled mafic component, as described here, may provide a basis for the identification of mafic components in mantle sources from other geodynamic environments, particularly mid-ocean ridges. □

Received 27 October 1995; accepted 1 July 1996.

- Allegre, C. J., Hart, S. R. & Minster, J. F. *Earth planet. Sci. Lett.* **66**, 191–213 (1983).
- White, W. M. *Geology* **13**, 115–118 (1985).
- Zindler, A. & Hart, S. R. *Rev. Earth planet. Sci.* **14**, 493–571 (1986).
- Hauri, E. H. & Hart, S. R. *Earth planet. Sci. Lett.* **114**, 353–371 (1993).
- Chase, C. G. *Earth planet. Sci. Lett.* **52**, 277–284 (1981).
- Devey, C. W. et al. *J. geophys. Res.* **95**, 5049–5066 (1990).
- Weaver, B. L. *Earth planet. Sci. Lett.* **104**, 381–397 (1991).
- White, W. M., McBirney, A. R. & Duncan, R. A. *J. geophys. Res.* **98**, 19533–19563 (1993).
- Duncan, R. A., Fisk, M. R., White, W. M. & Nielsen, R. L. *J. geophys. Res.* **99**, 24341–24357 (1994).
- Hofmann, A. W. *Earth planet. Sci. Lett.* **90**, 297–314 (1988).
- Jagoutz, E. et al. *Proc. lunar planet. Sci. Conf.* **10**, 2031–2050 (1979).
- Reisberg, L. & Zindler, A. *Earth planet. Sci. Lett.* **81**, 29–45 (1986).
- Roden, M. F., Irving, A. J. & Murthy, V. R. *Geochim. cosmochim. Acta* **52**, 461–473 (1988).
- Ionov, D. A., Kramm, U. & Stosch, H.-G. *Contr. Miner. Petrol.* **111**, 235–247 (1992).
- Ionov, D. A., Ashchepkov, I. V., Stosch, H.-G., Witt-Eickchen, G. & Seck, H. A. *J. Petrol.* **34**, 1141–1175 (1993).
- Blusztajn, J. & Shimizu, N. *Chem. Geol.* **111**, 227–243 (1994).
- Frey, F. A., Suen, J. & Stockman, H. W. *Geochim. cosmochim. Acta* **49**, 2469–2491 (1985).
- Hauri, E. H., Shimizu, N., Dieu, J. J. & Hart, S. R. *Nature* **356**, 221–227 (1993).
- Hauri, E. H. & Hart, S. R. *J. geophys. Res.* **99**, 24301–24321 (1994).
- Kurz, M. D., Jenkins, W. J., Hart, S. R. & Clague, D. A. *Earth planet. Sci. Lett.* **66**, 388–406 (1983).
- Frey, F. A. & Clague, D. A. *Earth planet. Sci. Lett.* **66**, 337–355 (1983).
- Staudigel, H. A. et al. *Earth planet. Sci. Lett.* **69**, 13–29 (1984).
- Garcia, M. O., Mahoney, J. J. & Ito, E. J. *geophys. Res.* **98**, 537–550 (1993).
- Garcia, M. O., Foss, D. J. P., West, H. B. & Mahoney, J. J. *J. Petrol.* **36**, 1647–1674 (1995).
- Hofmann, A. W., Feigenson, M. D. & Raczek, I. *Contr. Miner. Petrol.* **88**, 24–35 (1984).
- Chen, C.-Y., Frey, F. A., Rhodes, J. M. & Easton, R. M. in *Earth Processes: Reading the Isotopic Code* 161–182 (Geophys. Monogr. 95, Am. Geophys. Union, Washington DC, 1996).
- Kurz, M. D. & Kammer, D. P. *Earth planet. Sci. Lett.* **103**, 257–269 (1991).
- Kurz, M. D., Kenna, T. C., Kammer, D. P., Rhodes, J. M. & Garcia, M. O. in *Mauna Loa—A Decade Volcano* 289–306 (Geophys. Monogr. 92, Am. Geophys. Union, Washington DC, 1995).
- Rhodes, J. M. & Hart, S. R. in *Mauna Loa—A Decade Volcano* 263–288 (Geophys. Monogr. 92, Am. Geophys. Union, Washington DC, 1995).
- Hauri, E. H. & Kurz, M. D. *Earth planet. Sci. Lett.* (in the press).
- Frey, F. A. et al. *J. geophys. Res.* **96**, 14347–14375 (1991).
- Yang, H.-J., Frey, F. A., Garcia, M. O. & Clague, D. A. *J. geophys. Res.* **99**, 15577–15594 (1994).
- Yang, H.-J., Frey, F. A., Rhodes, J. M. & Garcia, M. O. *J. geophys. Res.* **101**, 11747–11768 (1996).
- Lassiter, J. C., DePaolo, D. J. & Tatsumoto, M. *J. geophys. Res.* **101**, 11769–11780 (1996).
- Rhodes, J. M. *J. geophys. Res.* **101**, 11729–11746 (1996).
- Feigenson, M. D., Hofmann, A. W. & Spera, F. J. *Contr. Miner. Petrol.* **84**, 390–405 (1983).
- Hofmann, A. W., Feigenson, M. D. & Raczek, I. *Contr. Miner. Petrol.* **95**, 114–122 (1987).
- Lanphere, M. A. & Frey, F. A. *Contr. Miner. Petrol.* **95**, 100–113 (1987).
- Fodor, R. V., Frey, F. A., Bauer, G. R. & Clague, D. A. *Contr. Miner. Petrol.* **110**, 442–462 (1992).
- Leeman, W. P., Gerlach, D. C., Garcia, M. O. & West, H. B. *Contr. Miner. Petrol.* **116**, 62–77 (1994).

- Chen, C. Y. & Frey, F. A. *J. geophys. Res.* **90**, 8743–8768 (1985).
- Kurz, M. D., Garcia, M. O., Frey, F. A. & O'Brien, P. A. *Geochim. cosmochim. Acta* **51**, 2905–2914 (1987).
- Chen, C.-Y., Frey, F. A., Garcia, M. O., Dalrymple, G. B. & Hart, S. R. *Contr. Miner. Petrol.* **106**, 183–200 (1991).
- Roden, M. F., Frey, F. A. & Clague, D. A. *Earth planet. Sci. Lett.* **69**, 141–158 (1984).
- Roden, M., Trull, T. W., Hart, S. R. & Frey, F. *Geochim. cosmochim. Acta* **58**, 1431–1440 (1994).
- Frey, F. A., Garcia, M. O. & Roden, M. F. *Geochim. cosmochim. Acta* **58**, 1441–1462 (1994).
- Bennett, V. C., Esat, T. M. & Norman, M. D. *Nature* **381**, 221–224 (1996).
- Frey, F. A. et al. *J. geophys. Res.* **95**, 1271–1300 (1990).
- Martin, C. E., Carlson, R. W., Shirey, S. B., Frey, F. A. & Chen, C.-Y. *Earth planet. Sci. Lett.* **128**, 287–301 (1994).
- Ford, C. E., Russell, D. G., Craven, J. A. & Fisk, M. R. *J. Petrol.* **24**, 256–265 (1983).
- Jackson, E. D., Silver, E. A. & Dalrymple, G. B. *Geol. Soc. Am. Bull.* **83**, 601–618 (1972).
- Hauri, E. H., Wagner, T. P. & Kurz, M. D. *Mineralog. Mag.* **58A**, 392–393 (1994).
- Hauri, E. H., Lassiter, J. C. & DePaolo, D. J. *J. geophys. Res.* **101**, 11793–11806 (1996).
- Kelemen, P. B., Dick, H. J. B. & Quick, J. E. *Nature* **345**, 521–524 (1992).
- Porcelli, D. R., O'Nions, R. K. & O'Reilly, S. Y. *Chem. Geol.* **54**, 237–249 (1986).
- Vance, D., Stone, J. O. H. & O'Nions, R. K. *Earth planet. Sci. Lett.* **96**, 147–160 (1989).
- Klein, F. W. & Koyanagi, R. Y. in *The Eastern Pacific Ocean and Hawaii, the Geology of North America* (eds Winterer, E. L., Hussong, D. M. & Decker, R. W.) 238–252 (Geol. Soc. Am., Boulder, CO, 1989).
- Dick, H. J. B., Fisher, R. L. & Bryan, W. B. *Earth planet. Sci. Lett.* **69**, 88–106 (1984).
- Klein, E. M. & Langmuir, C. H. *J. geophys. Res.* **92**, 8089–8115 (1987).
- Klein, E. M. & Langmuir, C. H. *J. geophys. Res.* **94**, 4241–4252 (1989).
- Hirose, K. & Kushiro, I. *Earth planet. Sci. Lett.* **114**, 477–489 (1993).
- Langmuir, C. H. & Hanson, G. N. *Phil. Trans. R. Soc. Lond.* **A297**, 383–407 (1980).
- Wilkinson, J. F. G. *Earth planet. Sci. Lett.* **75**, 129–138 (1985).
- Francis, D. *Lithos* **34**, 89–105 (1995).
- Arth, J. G., Barker, F., Peterman, Z. E. & Friedman, I. *J. Petrol.* **19**, 289–316 (1978).
- Drummond, M. S. & Defant, M. J. *J. geophys. Res.* **95**, 21503–21521 (1990).
- Rapp, R. P., Watson, E. B. & Miller, C. F. *Precamb. Res.* **51**, 1–25 (1991).
- Ghiorsso, M. S. & Sack, R. O. *Contr. Miner. Petrol.* **119**, 197–212 (1995).
- Hirschmann, M. M. & Stolper, E. M. *Contr. Miner. Petrol.* (in the press).
- Frey, F. A. & Rhodes, J. M. *Phil. Trans. R. Soc. Lond.* **A342**, 121–136 (1993).
- Agee, C. & Walker, D. *Earth planet. Sci. Lett.* **90**, 144–156 (1988).
- Wetherill, G. A. *Rev. Earth planet. Sci.* **18**, 205–256 (1990).
- Kato, T., Ringwood, A. E. & Inifune, T. *Earth planet. Sci. Lett.* **90**, 65–68 (1988).
- Baker, M. B. & Stolper, E. M. *Geochim. cosmochim. Acta* **58**, 2811–2827 (1994).
- Longhi, J. *Geochim. cosmochim. Acta* **59**, 2375–2386 (1995).
- Takahashi, E., Shimazaki, T., Tsuzaki, Y. & Yoshida, H. *Phil. Trans. R. Soc. Lond.* **A342**, 105–120 (1993).
- Walter, M. J. & Bertka, C. M. (abstr.) *Eos* **75**, 192 (1994).
- Herzberg, C. & Zhang, J. *J. geophys. Res.* (in the press).
- Hauri, E. H., Whitehead, J. A. & Hart, S. R. *J. geophys. Res.* **99**, 24275–24300 (1994).

ACKNOWLEDGEMENTS. Credit goes to M. Hirschmann for kindling the interest of many in pyroxenite melting; T. Wagner, M. Kurz, F. Frey and J. Lassiter for preprints and discussions; F. Frey, M. Garcia and P. Kelemen for comments on an early draft; and S. Eggins and W. White for reviews. This work was supported by the US NSF.

CORRESPONDENCE should be addressed to E.H.H. (e-mail: hauri@dtm.ciw.edu).

Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts

Rebecca Heald*, Régis Tournebize*, Thiemo Blank†, Raphael Sandaltzopoulos†, Peter Becker†, Anthony Hyman* & Eric Karsenti*

* Mitotic Spindle Group in the Cell Biology Program, † Gene Expression Program, EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Functional nuclei and mitotic spindles are shown to assemble around DNA-coated beads incubated in *Xenopus* egg extracts. Bipolar spindles assemble in the absence of centrosomes and kinetochores, indicating that bipolarity is an intrinsic property of microtubules assembling around chromatin in a mitotic cytoplasm. Microtubules nucleated at dispersed sites with random polarity rearrange into two arrays of uniform polarity. Spindle-pole formation requires cytoplasmic dynein-dependent translocation of microtubules across one another. It is proposed that spindles form in the absence of centrosomes by motor-dependent sorting of microtubules according to their polarity.

THE mitotic spindle is the apparatus with which eukaryotic cells segregate their replicated chromosomes during cell division. An assembled spindle consists of microtubules extending from two poles to the chromosomes at the centre of the spindle. Some microtubules attach to chromosomes, which others overlap in the centre of the spindle, creating the typical bipolar shape. The slow-growing, minus ends of microtubules are oriented towards the poles, and the fast-growing, plus ends towards chromatin^{1,2}. Organization of microtubules into two poles of uniform polarity is required for the process of chromosome segregation, which occurs by the transport of the duplicated chromatids to each spindle pole during anaphase. Thus, the mechanisms that establish microtubule orientation and bipolarity are central to the process of cell division.

In the most generally recognized model of spindle assembly, dynamic microtubules nucleated with their minus ends at two centrosomes are captured and stabilized when their plus ends encounter kinetochores, the specialized protein complexes that assemble onto centromeric DNA^{3,4}. In this 'search and capture' model, bipolarity and microtubule orientation are imposed by the presence of two microtubule-nucleating sites such as centrosomes. However, the generality of this mechanism is challenged by several observations⁵. During mitosis in plants and meiosis of most animal species, microtubules growing from multiple sites around condensed chromatin self-organize into a spindle in the absence of centrosomes or discrete microtubule-organizing centres^{6–10}. Therefore, two distinct microtubule nucleation sites are not always a prerequisite for spindle bipolarity. An obligate role for kinetochores is also challenged by several experiments. Prokaryotic DNA lacking centromeric sequences induces the assembly of microtubule arrays when microinjected into *Xenopus* eggs¹¹. Spindles without kinetochores can assemble in extracts of *Xenopus* eggs¹². Finally, chromosome arms will stabilize microtubules and influence mitotic-spindle assembly independently of kinetochores, as shown by micromanipulation studies in insect cells¹³.

The 'search and capture' mechanism thus appears to be insufficient to explain bipolar spindle assembly in systems other than vertebrate somatic cells. Moreover, the respective roles of centrosomes, chromosomes and kinetochores in this process remain controversial. To study the relative contributions of spindle components and to investigate general mechanisms of bipolar

spindle assembly, we have developed an *in vitro* system in which magnetic beads coupled with plasmid DNA act as artificial chromosomes in *Xenopus* egg extracts. Using this system, we find that spindle assembly does not require centrosomes or centromeric DNA sequences. Rather, mitotic chromatin itself is the driving force for microtubule organization into bipolar spindles.

Using polarity-marked microtubules to follow microtubule movements, we show that bipolarity is generated by the self-organization of randomly growing microtubules into two anti-parallel arrays. Finally, we show that at least one microtubule-based motor, cytoplasmic dynein, is required for the formation of spindle poles.

Spindle assembly without bipolar cues

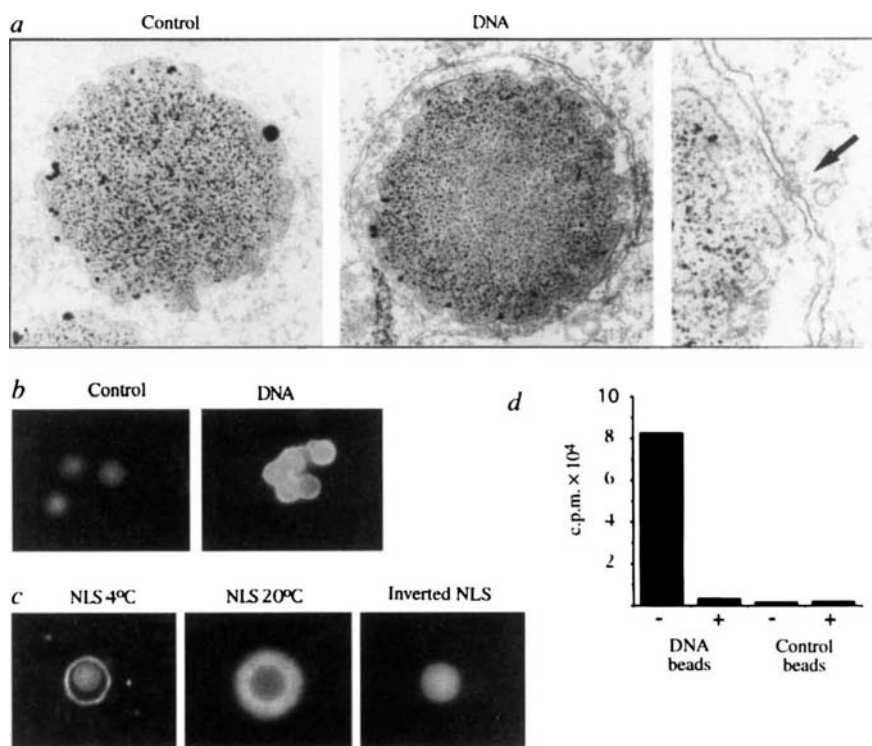
To generate a well defined source of chromatin with which to study the pathway of spindle assembly, we linked plasmid DNA to magnetic beads¹⁴. Each bead was coated with 0.3 pg DNA, about one tenth the amount of DNA found in a *Xenopus* sperm nucleus¹⁵. Unlike sperm nuclei, DNA attached to these beads contains no centromeric DNA and therefore cannot assemble kinetochores. DNA linked to beads assembles into chromatin when incubated in *Xenopus* egg extracts, as indicated by the binding of specific chromatin proteins and nucleosome assembly¹⁴ (data not shown).

We examined the functional characteristics of chromatin beads after incubation in interphase extracts. Electron microscopy revealed a double membrane, which contained constrictions with the dimensions and morphology of nuclear pores, around chromatin beads, but not control beads (Fig. 1a). Immunofluorescent staining of the *Xenopus* LIII lamin confirmed the assembly of a nuclear lamina (Fig. 1b)¹⁶. A fluorescent protein substrate modified with a nuclear localization sequence (NLS) bound to bead nuclei at 4 °C and was transported at 20 °C, whereas the reverse NLS substrate was excluded, demonstrating the presence of functional nuclear pores (Fig. 1c)¹⁷. Chromatin bead nuclei incorporated ³²P-dCTP, indicating that DNA linked to beads is replicated (Fig. 1d). Thus DNA coupled to beads induces the assembly of functional nuclei and replicates. As chromatin beads are easily retrievable with a magnet, this system provides a powerful tool for biochemical studies of nuclear processes.

To investigate whether chromatin beads can induce spindle assembly, beads were first incubated in an interphase extract to

FIG. 1 Functional nuclei form around chromatin beads in interphase. *a*, Electron micrograph of control and chromatin bead. Inset, arrow indicates nuclear pore. *b*, Immunofluorescent staining of *Xenopus* LIII lamin of control and chromatin beads. *c*, Nuclear transport assay of chromatin beads incubated with nuclear localization sequence (NLS) coupled to BSA at 4 °C, 20 °C, or with reverse NLS-BSA at 20 °C. *d*, ³²P-dCTP incorporation by chromatin and control beads in the absence (–) and presence (+) of aphidicolin, a specific inhibitor of DNA replication, but not repair. Scale: bead diameter is 2.8 µm.

METHODS. Bluescript plasmid containing a 5-kb insert of non-coding *Drosophila* DNA was linearized, biotinylated and coupled to streptavidin magnetic beads in a reaction containing 50 mM Tris-HCl, pH 8, 1 M NaCl, 2 mM EDTA, and an immobilization activator available from Dynal, Oslo¹⁴. Coupling efficiency was routinely 20 µg DNA per 1 mg dynabeads. 10,000g cytoplasmic extracts were prepared from unfertilized *Xenopus* eggs arrested in metaphase of meiosis II by cytostatic-factor (CSF) activity, termed mitotic extracts¹², as described³¹. Interphase extracts were prepared from mitotic extracts by addition of 0.4 mM calcium and 200 µg ml^{–1} cycloheximide and a 40-min incubation at 20 °C. For nuclear assembly experiments, control or DNA beads were incubated for 3 h in interphase extracts at 20 °C at a ratio of 0.5 µg DNA beads or equivalent uncoupled beads per 100-µl extract. Beads were fixed for electron microscopy with 10 volumes of 1% glutaraldehyde in PBS for 1 h, then pellets were embedded in Epon, sectioned and observed with a Phillips 300 microscope. For immunofluorescence, 15 µl of bead nuclear assembly reactions were fixed with 1 ml 0.25% glutaraldehyde in BRB80 (80 mM PIPES, pH 6.8, 1 mM MgCl₂, 1 mM EGTA) containing 30% glycerol and 1% Triton-X100, then spun onto coverslips through a 40% glycerol/BRB80 cushion in modified Corex tubes as described³². Coverslips were post-fixed in –20 °C acetone for 5 min, then processed for immunofluorescence with the *Xenopus* LIII lamin antibody. For nuclear transport assays, fluorescein-labelled protein conjugates, consisting of BSA coupled either to the



functional SV40 NLS or to the inverted peptide (inverted NLS), were added to nuclear assembly reactions after 2.5 h to 75 µg ml^{–1} (ref. 17). After 30 min, samples were fixed with PBS/0.5% glutaraldehyde, spun onto coverslips, and viewed by confocal microscopy. For the DNA replication assay, 0.125 mg DNA beads or an equivalent amount of control beads was incubated in 30 µl interphase extract for 2 h in the presence of 5 µCi of ³²P-dCTP, with or without 20 µg ml^{–1} aphidicolin. Beads were retrieved and washed extensively with 10 mM HEPES, pH 7.6, 3 mM MgCl₂, 0.5 mM EGTA and 0.05% Triton-X100, then transferred to vials and counted by scintillation.

allow chromatin formation and nuclear assembly, then retrieved and resuspended in a mitotic extract. Fluorochrome-labelled tubulin was added to the extract to label the microtubules. Within 90 min, spindle-like structures assembled around clusters of chromatin beads (Fig. 2), which no longer had nuclear envelopes (data not shown). To address whether DNA is specifically required for spindle assembly, we tested beads that had been coupled with similar concentrations of another acidic polymer, polyglutamic acid. These beads failed to promote microtubule growth or spindle assembly (data not shown). We compared the morphology of chromatin bead spindles with those assembled around *Xenopus* sperm, during which the centrosome associated with the sperm nucleus duplicates, and the two centrosomes become the spindle poles¹⁸. Like sperm spindles, chromatin bead spindles are bipolar structures with two well focused poles, and have a similar distribution of spindle lengths (Fig. 2a). As there are no centrosomes present in frog eggs or egg extracts, the bipolar spindles formed around chromatin beads have assembled in the absence of centrosomes.

Because the plasmid DNA coupled to beads contains no centromeres, chromatin beads contain no kinetochores. Nevertheless, like sperm DNA, chromatin beads were localized in the centre of bipolar microtubule arrays, often aligned in a row, mimicking a metaphase plate. Of the spindle arrays formed, 90% were arranged in a bipolar configuration after 90 min (Fig. 2b), a percentage similar to that found for sperm DNA spindles in the same extracts (data not shown).

Thus, chromatin beads specifically induce the assembly of spindles that are morphologically indistinguishable from spindles

formed around sperm chromatin. In this system, two possible cues for bipolarity, duplicated centrosomes and duplicated kinetochores, are not necessary for bipolar spindle assembly.

Self-organization of microtubules

To analyse the mechanism of spindle assembly in the absence of centrosomes, we examined intermediates formed during the process (Fig. 3). The first microtubule structures associated with chromatin beads were visible after 10–20 min of incubation in a mitotic extract. Microtubules grew from one or more sites on or near the beads, often forming arrays extending radially. We define this stage as 'nucleation'. By 30–45 min of incubation, most microtubules had rearranged into dense bundles, tightly packed around the beads. We define this stage as 'coalescence'. At 60 min and onwards, bipolar spindles were increasingly apparent. Clearly, these spindles are not assembling by a 'search and capture' mechanism, in which microtubules nucleated at centrosomes are captured and stabilized by chromatin. How then can microtubules apparently nucleated in random orientations become rearranged into a bipolar array?

One possibility is that spindles assemble around chromatin beads by movement of the nucleated microtubules. Indeed, microtubule-based motors are known to function in the process of spindle formation¹⁹, and can generate polarized arrays of microtubules²⁰. Unfortunately, the density of microtubules during spindle formation prevents observation of individual microtubules. Furthermore, the orientation of microtubules during the successive phases of assembly could not be determined. We therefore decided to add preformed stable polarity-marked

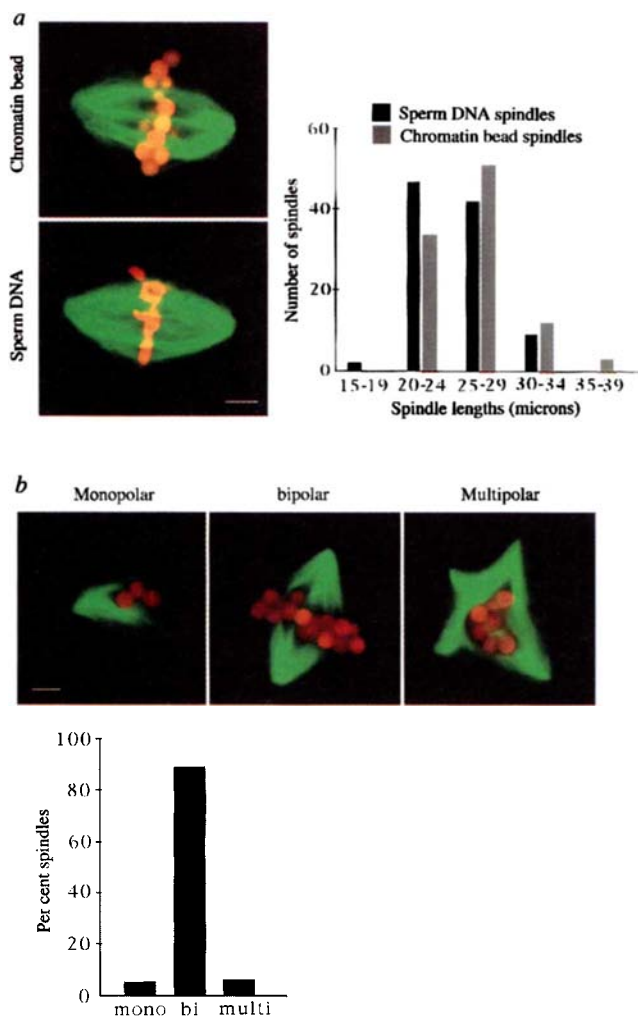


FIG. 2 Spindles assemble around chromatin beads in mitosis. **a**, Left, spindle assembled around chromatin beads and wild-type spindle assembled around sperm DNA; right, distribution of 100 spindle lengths. **b**, Frequency of chromatin bead spindle morphologies among 635 spindles and an example of monopolar, bipolar and multipolar structures. Scale bars in **a** and **b**, 5 μ m.

METHODS. Mitotic spindles were assembled around demembrated sperm nuclei by the interphase to mitotic pathway as described¹². To assemble chromatin bead spindles, 0.6 μ g DNA or equivalent control beads were incubated in 100 μ l interphase extract for 2 h at 20 °C before addition of 0.5 volumes of fresh mitotic extract. After an additional 30-min incubation, beads were retrieved on ice and resuspended in 200 μ l fresh mitotic extract containing 0.2 mg ml⁻¹ fluorochrome-labelled tubulin. Rhodamine- or fluorescein-labelled bovine brain tubulin was prepared as described³³. Spindle-assembly reactions incubated at 20 °C for 60–90 min were monitored by fixing small aliquots of the reaction as described¹². The clustering or aggregation of chromatin beads is a result of chromatin assembly, which increases the stickiness of DNA. The time necessary for chromatin bead spindle assembly, and the percentage of spindles formed, depended on the extract. Spindle assembly around chromatin beads does not require CSF activity, because spindles also assembled around chromatin beads incubated in interphase extracts induced to enter mitosis by addition of cyclin B. For data acquisition, spindles assembled in the presence of fluorescein-tubulin were diluted and spun onto coverslips as described¹². DNA was stained with propidium iodide. Samples were viewed with a fluorescent or confocal microscope.

minus ends leading (Fig. 5c). Thus, preformed microtubules associate with and translocate over spindle microtubules with their minus ends leading, suggesting that seeds act as markers of microtubule polarity and movements during spindle formation. The uniform orientation and direction of seed movement on spindles indicates the establishment of uniform polarity of microtubules clustered at each pole.

We next examined the behaviour of polarity-marked seeds during spindle assembly around chromatin beads. At all stages of spindle assembly, when polarity could be unequivocally determined, at least 90% of seeds moved along microtubules with their minus ends leading at an average rate of 6 μ m per min, suggesting that a common motor was active (data not shown). During the nucleation phase, seeds moved in all directions on growing microtubules, both towards and away from the chromatin beads (Fig. 5a). To quantify seed movement during nucleation, we divided the array into four quadrants and scored seed orientation and direction of movement. The number of seeds moving into each quadrant was approximately equal. During the coalescence stage, a large bundle of microtubules was often apparent, defining

microtubules to spindles during the various stages of assembly. We reasoned that such microtubules would become associated with microtubule arrays and behave as tracers for microtubule movements and polarity during spindle morphogenesis. Fluorescent stable microtubule 'seeds' were constructed, with a dimly labelled plus end and a bright minus end²¹.

We first tested whether preformed microtubules could associate with spindles. Spindles formed around chromatin beads in *Xenopus* egg extracts were visualized by addition of fluorescein-labelled tubulin. Rhodamine-labelled microtubule seeds were then added to the reaction and viewed by video microscopy. Seeds bound to spindle microtubules and moved polewards, where they accumulated (Fig. 4a). Seeds moved in a saltatory manner, at an average rate of 6 μ m per min (Fig. 4b), with their

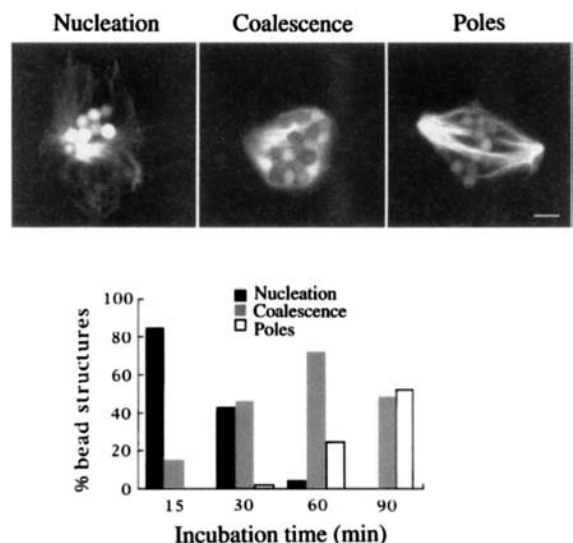


FIG. 3 Top, chromatin bead spindle-assembly intermediates, showing nucleation, coalescence and spindle pole structures. Scale bar, 5 μ m; bottom, time course of spindle assembly showing the percentage of intermediates scored at 15, 30, 60 and 90 min.

METHODS. Chromatin bead spindle-assembly reactions were monitored after preincubated beads were resuspended in fresh CSF extract. At various time during spindle assembly, aliquots were fixed and squashed or spun onto coverslips, and analysed by fluorescence microscopy. The efficiency of chromatin bead spindle assembly varied from extract to extract, with the final percentage of spindles ranging from 50 to 90%. In the absence of bead preincubation, less than 5% of spindles were apparent after 90 min incubation (not shown).

a single axis of microtubules. Seed movement occurred preferentially along this axis, in either direction (Fig. 5b). In some cases, multiple bundles of microtubule arrays were present around the chromatin beads. Seeds translocated along bundles in either direction, and did not accumulate at particular sites. Finally, after the establishment of bipolarity, seeds moved towards spindle poles as already described (Fig. 5c). These observations indicate that microtubules nucleate around chromatin beads with random polarity and subsequently coalesce into bundles of mixed polarity. From these bundles, poles emerge which have uniform polarity. This seems to occur through microtubule translocation.

Dynein organizes spindle poles

Seed movement with the minus end leading could, in principle, be due to either plus-end-directed motors fixed to spindle microtubules, or minus-end-directed motors fixed to the seeds. To distinguish between these possibilities, we added inhibitors of motor proteins to preformed spindles (Fig. 6a). Addition of 2.5 mM AMP-PNP, an inhibitor of kinesin-like proteins²², did not inhibit seed translocation and the accumulation of seeds at spindle poles, whereas 100 μ M vanadate, an inhibitor of cytoplasmic dynein²³, severely reduced the number of seeds bound to spindles (Fig. 6a). Those seeds that bound to spindles in the presence of vanadate did not move (data not shown). To confirm the role of dynein, we tested the effects of a monoclonal IgM antibody, 70.1, which recognizes the intermediate chain of chicken

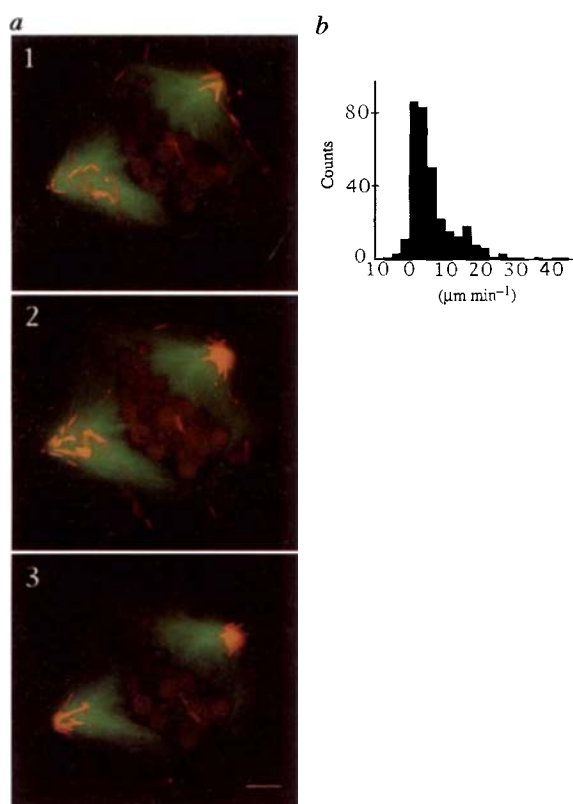


FIG. 4 Direction and rate of seed movements. **a**, Three successive frames showing the translocation of preformed stable microtubules to the poles of chromatin bead spindle. Scale bar, 5 μm . **b**, Rate of seed movement. METHODS. Microtubule seeds were polymerized from 5 μM rhodamine-labelled tubulin in the presence of 0.1 mM GMP-CPP, a non-hydrolysable GTP analogue, in BRB80 for 30 min at 37 $^{\circ}\text{C}$, creating stable microtubules 2–4 μm long. Before use, seeds were spun for 5 min at 70,000 g and resuspended in BRB80. After addition of seeds, spindles were observed by video microscopy using a 3-chip colour camera (Photonic Sciences). Rates of seed movement were measured over 5-s intervals.

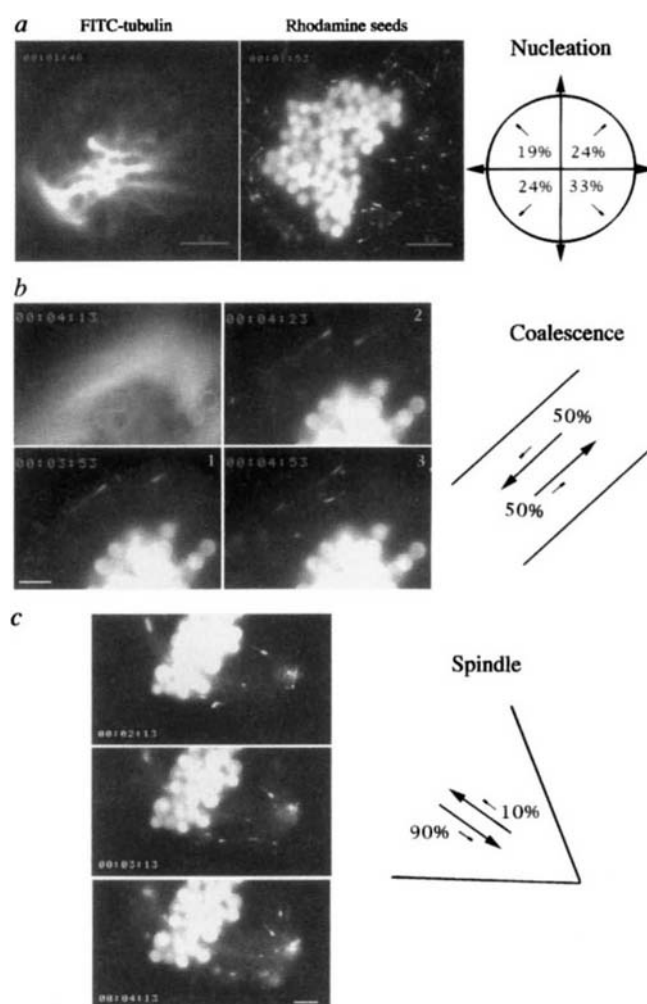


FIG. 5 Polarity-marked seed movement and orientation during spindle assembly around chromatin beads. **a**, Nucleation: polarity-marked microtubule seeds move randomly on microtubules nucleated around chromatin beads. Diagram on the right shows the structure divided into quadrants. Each seed was scored according to which of the four directions it moved during the recording. **b**, Coalescence: bidirectional seed movements on microtubule bundles coalescing around chromatin beads. Upper-left frame is the microtubule bundle; the sequence of seed movement is labelled 1–3. Two seeds can be seen moving past each other, indicating opposite microtubule polarity in bundles. To the right is shown the percentage of seeds translocating in each direction, minus end first; right, percentage of seeds translocating towards and away from the pole. Scale bars: **a**, 8 μm ; **b**, **c** 5 μm .

METHODS. Polarity-marked seeds were prepared by 30-min incubation of rhodamine CMP-CPP microtubules in 3 μM rhodamine tubulin diluted 1:10 with unlabelled tubulin. Minus-end growth was blocked by addition of 1.5 μM NEM tubulin³³. The dimly labelled plus-ends were between 4–8 μm long. Polarity-marked seeds were added to chromatin bead spindle assembly reactions and immediately observed by video microscopy. Saturated haemoglobin was added at a dilution of 1:20 to inhibit photobleaching³⁴. Seed movement data was acquired using NIH image. For data analysis during the nucleation stage, 30 polarity marked seeds were scored for their orientation and direction of movement on a representative chromatin bead/microtubule array. During coalescence, 5 pairs of seeds on 5 different arrays were observed moving past one another along microtubule bundles. After pole formation, 100 seeds on 70 different spindles were scored for movement towards the near or far pole.

cytoplasmic dynein and crossreacts with the *Xenopus* homologue²⁴. Addition of antibody 70.1 to 1 mg ml^{-1} to spindles completely blocked seed translocation, whereas control IgM had no effect. Like vanadate, antibody 70.1 reduced the number of seeds bound and blocked their movement. Similarly, addition of the antibody prevented seed translocation at all stages in spindle assembly (data not shown). Because all seed movement appears to be due to a minus-end-directed motor, these results confirm that seed movement can be used as a marker of endogenous polarity of microtubules in spindles.

To assess the role of dynein-dependent microtubule motility in spindle formation, spindle assembly was initiated around chromatin beads in extracts preincubated with antibody 70.1 (Fig. 6b). All of the spindle arrays formed contained parallel bundles of microtubules, centred around the beads. However, in more than 200 spindles examined, spindle poles never appeared. The microtubule arrays ended in frayed, unfocused structures, wider than the poles of spindles assembled in the presence of control antibodies (Fig. 6c). Similarly, addition of inhibitors of dynein to preformed spindles caused a gradual dissolution of pole structures

into parallel bundles (Fig. 6a). These results show that dynein is required for focusing microtubules into poles, but not for bundling into longitudinal arrays. As microtubule translocation also required dynein, these data indicate that minus-end-directed movement of microtubules may be required for both spindle-pole assembly and to maintain pole stability. However, dynein-dependent microtubule translocation is not required for the formation of parallel arrays of microtubules around chromatin, which defines the bipolar axis of the spindle.

Discussion

We have developed an *in vitro* system in which beads coated with DNA function physiologically as artificial chromosomes both in interphase and mitotic extracts of *Xenopus* eggs. Chromatin beads induce the assembly of functional nuclei in interphase, competent to transport nuclear substrates and replicate DNA. In mitosis, chromatin beads direct the assembly of bipolar spindles in the absence of discrete microtubule-nucleating centres and centromeric DNA sequences. These results demonstrate that chromatin is sufficient to direct spindle assembly, and that bipolarity is intrinsic to microtubule self-assemblies around mitotic chromatin. Owing to their paired nature, duplicated centrosomes or sister kinetochores can both promote spindle bipolarity. We show, however, that chromatin bead spindles are predominantly bipolar in the absence of these cues.

We have used polarity-marked microtubules to examine the morphogenetic movements of microtubules during assembly of bipolar spindles around chromatin beads. The polarity-marked microtubules serve two purposes. First, because they translocate across other microtubules with their minus ends leading by using dynein, they serve as markers for the endogenous polarity of microtubules in spindles. Second, they allow us to assess the role of this minus-end-directed motility during spindle assembly. We show that sorting of microtubule minus ends into poles, but not reorganization of microtubules into a parallel bundle, requires dynein-dependent sliding of microtubules.

The assembly of a spindle in the absence of centrosomes raises two important questions about the mechanism of spindle assembly. How do two poles form, and how do the microtubules in these two poles acquire uniform polarity? We present a model for spindle assembly based on our observations (Fig. 7). Although presented

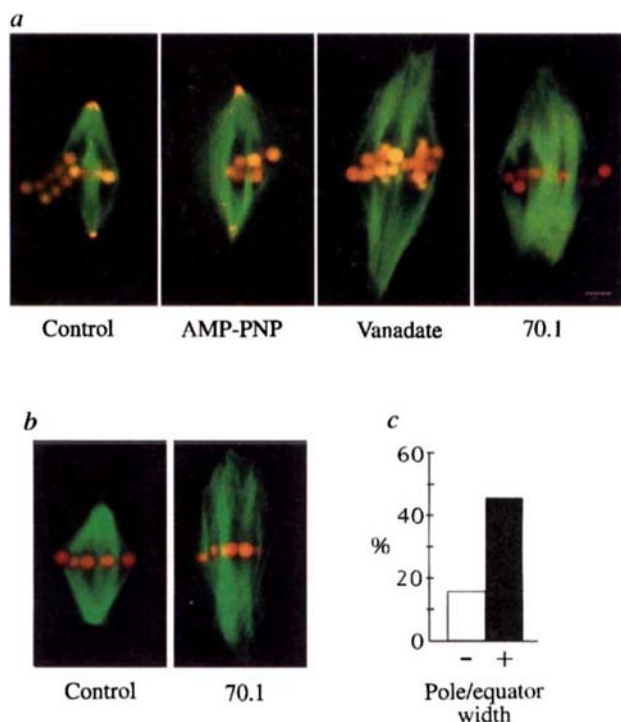


FIG. 6 Dynein is required for microtubule translocation and chromatin bead spindle-pole assembly and stability. **a**, Seed localization on preformed chromatin bead spindles after addition of control buffer, 2.5 mM AMP-PNP, 100 μM vanadate, or 1 mg ml^{-1} 70.1 monoclonal anti-dynein intermediate chain antibody. Scale bar, 5 μm . **b**, Chromatin bead spindles assembled in the presence of control or 70.1 antibodies. **c**, Pole width relative to spindle equator width of 100 chromatin bead spindles assembled in the presence of control (-) or 70.1 antibody (+).

METHODS. Nucleotide analogues were added as magnesium salts at a 1:10 dilution immediately before seed addition to chromatin bead spindles. After 5 min, reactions were diluted 1:10 with 30% glycerol/BRB80/1% Triton X100 and spun onto coverslips. Anti-dynein intermediate-chain 70.1 and control anti-IgG4 monoclonal antibodies (both IgM ascites) were dialysed against 50 mM potassium acetate, 0.5 mM MgCl_2 , then concentrated to 10 mg ml^{-1} . Antibodies were added at a ratio of 1:10 to chromatin bead spindles, which were diluted and spun onto coverslips after 10 min. For spindle assembly in the presence of antibodies, extracts were preincubated for 30 min on ice with 1 mg ml^{-1} 70.1 or control ascites. Chromatin beads were added and incubated for 90 min at 20°C , then diluted and spun onto coverslips. Chromatin beads were stained with propidium iodide and samples observed with a confocal microscope.

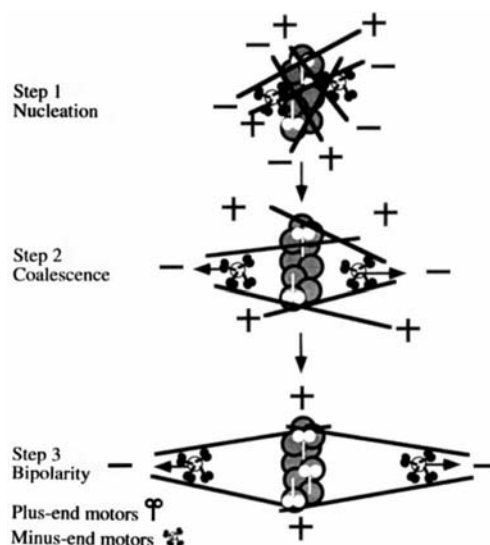


FIG. 7 Model of bipolar spindle assembly around chromatin beads. Step 1 shows microtubules nucleated around chromatin, with random polarity with respect to the chromatin beads. In step 2, the microtubules form a longitudinal bundle, and have been clustered together at their minus ends by dynein. In step 3, the clusters of microtubule minus ends are moved away by plus-end-directed motors bound to the chromatin.

as distinct steps, these processes seem to overlap in time. In the first step, microtubules appear to grow randomly close to the chromatin beads, in all orientations. We do not know how chromatin induces microtubule nucleation. One possibility is that chromatin acts as a nucleation site for microtubules, but this would result in a focus of minus ends on the beads, instead of the randomly oriented microtubules we observe. A more likely explanation is that chromatin changes the local state of the cytoplasm to favour microtubule nucleation and stabilization²⁵.

Next, microtubules of mixed polarity coalesce into bundles. The preferential alignment of microtubules into bundles may be promoted by non-covalent microtubule crosslinking, by either microtubule-associated proteins or microtubule-based motors, both of which have been implicated in spindle assembly^{19,26}. In this step, microtubules are limited to two orientations relative to one another, parallel or antiparallel. Consequently, a single axis of microtubules is established, which defines the orientation of the bipolar array. This explains why bipolarity predominates in our experiments: it simply derives from the physical properties of linear polymers that tend to align into a bundle when crosslinked by some activity.

In the third step, microtubule minus ends focus together, combining microtubules of the same polarity within the bundle. This requires dynein-dependent microtubule sliding. A mechanism by which dynein may generate polar arrays of microtubules has already been described²⁷. Dynein oligomers crosslink neighbouring microtubules could move them along each other, creating a focus of minus ends.

In the final step of spindle assembly around chromatin beads, two microtubule arrays of uniform polarity emerge at the same time as poles become focused and extend away from the chromatin beads. The extension of poles may be the result of plus-end-directed motors associated with chromatin²⁸, of microtubule

growth, or both. Thus, this model proposes that motor-dependent sorting of microtubules into arrays of uniform polarity is an important step in spindle assembly.

Our results also have implications for the mechanism by which chromosomes are balanced on the metaphase plate. Indeed, this is thought to depend upon a balance of forces pulling kinetochores towards the poles and pushing chromosome arms away from the poles. As spindles formed around chromatin beads lack proper kinetochores, our results indicate that pushing forces acting on chromatin can generate a bipolar symmetrical array of microtubules in the absence of pulling forces applied on kinetochores.

The model we have developed here differs from an earlier one³ in which dynamic microtubules nucleated from centrosomes act as searching devices that are captured and stabilized by kinetochores. In this model³, bipolarity is provided by the existence of two centrosomes and paired kinetochores. In our model, bipolarity arises simply from the asymmetry introduced in the cytoplasm by chromatin, the intrinsic polarity of microtubules, and the activity of motors that crosslink and read this polarity.

Meiotic spindles assemble according to the mechanism suggested by this model, but we believe that similar principles apply in mitosis⁵. For instance, a general requirement for dynein in spindle assembly is supported by its localization to spindle poles²⁹, and the disruption of spindle assembly in somatic cells by overexpression of p50, a component of dynactin, a dynein-binding complex³⁰. If centrosomes and kinetochores are not essential to establish spindle polarity, what is their function? We believe that the real function of centrosomes is to provide a link, through astral microtubules, between spindle poles and cues present in the cytoplasm or at the cell cortex. This link would be essential to determine spindle orientation in multicellular organisms. The most obvious function of kinetochores is in chromosome segregation during anaphase. □

Received 12 April; accepted 28 June 1996.

1. Telzer, B. R. & Haimo, L. T. *J. Cell Biol.* **89**, 373–378 (1981).
2. McIntosh, J. R. & Euteneuer, U. *J. Cell Biol.* **98**, 525–533 (1984).
3. Kirschner, M. W. & Mitchison, T. J. *Cell* **45**, 329–342 (1986).
4. Inoue, S. & Salmon, E. D. *Molec. cell. Biol.* **6**, 1619–1640 (1995).
5. Hyman, A. A. & Karsenti, E. *Cell* **84**, 401–411 (1996).
6. Theurkauf, W. E. & Hawley, R. A. *J. Cell Biol.* **116**, 1167–1180 (1992).
7. Gard, D. L. *Dev. Biol.* **151**, 516–530 (1992).
8. Lambert, A. M. & Lloyd, C. W. in *Microtubules* (eds Hyams, J. & Lloyd, C. W.) 325–342 (Wiley-Liss, New York, 1994).
9. Bajer, A. S. & Mole Bajer, J. *Cold Spring Harb. Symp. quant. Biol.* **46**, 263–283 (1982).
10. Albertson, D. G. & Thomson, J. N. *Chromosome Res.* **1**, 15–26 (1993).
11. Karsenti, E., Newport, J. & Kirschner, M. J. *Cell Biol.* **99**, 475–575 (1984).
12. Sawin, K. E. & Mitchison, T. J. *J. Cell Biol.* **112**, 925–940 (1990).
13. Zhang, D. & Nicklas, R. B. *J. Cell Biol.* **129**, 1287–1300 (1995).
14. Sandaltzopoulos, R., Blank, T. & Becker, P. B. *EMBO J.* **13**, 373–379 (1994).
15. Swanson, C. P., Merz, T. & Young, W. J. *Cytogenetics* 494–496 (Prentice Hall, Englewood Cliffs, 1981).
16. Lourim, D. & Krohne, G. *J. Cell Biol.* **123**, 501–512 (1993).
17. Weis, K., Mattaji, I. W. & Lamond, A. I. *Science* **268**, 1049–1053 (1995).
18. Gard, D. L., Hafezi, S., Zhang, T. & Doxsey, S. J. *J. Cell Biol.* **110**, 2033–2042 (1990).
19. Vemos, I. & Karsenti, E. *Curr. Opin. Cell Biol.* **8**, 4–9 (1996).
20. Urrutia, R., McNiven, M. A., Albanesi, J. P., Murphy, D. B. & Kachar, B. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6701–6705 (1991).

21. Hyman, A. A. *J. Cell Sci.* (suppl.) **14**, 125–127 (1991).
22. Vale, R. D., Reese, T. S. & Sheetz, M. P. *Cell* **42**, 39–50 (1985).
23. Shpetner, H. S., Paschal, B. M. & Vallee, R. B. *J. Cell Biol.* **107**, 1001–1009 (1988).
24. Steuer, E. R., Wordeman, L., Schroer, T. A. & Sheetz, M. P. *Nature* **345**, 266–268 (1990).
25. Dogterom, M., Felix, M.-A. & Guet, C. C. & Liebler, S. J. *Cell Biol.* **133**, 125–140 (1996).
26. Pellman, D., Bagget, M., Tu, H. & Fink, G. R. *J. Cell Biol.* **130**, 1373–1385 (1995).
27. Verde, F., Berrez, J. M., Antony, C. & Karsenti, E. *J. Cell Biol.* **112**, 1177–1187 (1991).
28. Vemos, I. & Karsenti, E. *Trends Cell Biol.* **5**, 297–301 (1995).
29. Pfarr, C. M. et al. *Nature* **345**, 263–265 (1990).
30. Echeverri, C. J., Paschal, B. M., Vaughan, K. T. & Vallee, R. B. *J. Cell Biol.* **132**, 617–634 (1996).
31. Murray, A. M. *Meth. Cell Biol.* **36**, 591–603 (1991).
32. Mitchison, T. J. a. K. & Kirschner, M. W. *Nature* **312**, 232–236 (1984).
33. Hyman, A. A. et al. *Meth. Enzym.* **196**, 478–485 (1991).
34. Verde, F., Dogterom, M., Stelzer, E., Karsenti, E. & Leibler, S. *J. Cell Biol.* **118**, 1097–1108 (1992).

ACKNOWLEDGEMENTS. We thank A. Haberman for assistance with electron microscopy; G. Krohne for anti-lamin LIII antibodies; I. Palacios for nuclear transport substrates; and S. Andersen, S. Eaton, M. Glotzer, P. Gönczy, S. Reinsch, I. Vemos and M. Way for critically reading the manuscript. This work was supported by a Human Frontier Science Program grant to E.K. and a Human Capital and Mobility Research Network grant to E.K. and A.H. R.H. was supported by a postdoctoral fellowship from the American Cancer Society.

CORRESPONDENCE and requests for materials should be addressed to R.H. (e-mail: Heald@EMBL-Heidelberg.de).

Detection of molecular gas in the quasar BR1202 – 0725 at redshift $z = 4.69$

K. Ohta^{*†}, T. Yamada[‡], K. Nakanishi^{*}, K. Kohno^{§||},
M. Akiyama^{*} & R. Kawabe[§]

^{*} Department of Astronomy, Kyoto University, Kyoto 606-01, Japan

[†] Institute for Astronomy, University of Hawaii, 2680 Woodlawn Drive, Honolulu, Hawaii 96822, USA

[‡] Cosmic Radiation Laboratory, The Institute of Physical and Chemical Research (RIKEN), Hirosawa Wako, Saitama 351-01, Japan

[§] Nobeyama Radio Observatory, National Astronomical Observatory, Minamimaki, Minamisaku, Nagano 384-13, Japan

^{||} Department of Astronomy, The University of Tokyo, Tokyo 113, Japan

ALTHOUGH great efforts^{1–8} have been made to locate molecular gas—the material out of which stars form—in the early Universe, there have been only two firm detections at high redshift. Both are gravitationally lensed objects at redshift $z \approx 2.5$ (refs 9–14). Here we report the detection of CO emission from the radio-quiet quasar BR1202 – 0725, which is at redshift $z = 4.69$. From the observed CO luminosity, we estimate that almost 10^{11} solar masses of molecular hydrogen are associated with the quasar; this is comparable to the stellar mass of a present-day luminous galaxy. Our results suggest that BR1202 – 0725 is a massive galaxy, in which the gas is largely concentrated in the central region, and that it is currently undergoing a large burst of star formation.

BR1202 – 0725 was found in an optical multi-colour based search¹⁵ and is the third-highest-redshift quasar known to date. Subsequent submillimetre observations^{16,17} revealed significant dust thermal emission towards this object. The amount of dust was estimated to be $\sim 10^9 M_\odot$, assuming a dust temperature of 50–100 K (ref. 17) which makes this object one of the most promising targets for the search of molecular gas in high-redshift objects, by considering a canonical gas-to-dust ratio of 100–500 in galaxies.

Aperture-synthesis observations of BR1202 – 0725 in the $^{12}\text{CO}(J = 5-4)$ emission line were made with the Nobeyama Millimeter Array (six 10-m antennas) during two separated periods. The instrument setup used and the observational techniques were basically the same as those described in Kawabe *et al.*¹⁰ when reporting the detection of CO($J = 3-2$) emission from the $z = 2.3$ galaxy IRAS F10214 + 4724. The spectrum was centred at 101.227 GHz, corresponding to a redshift of 4.693 for the CO($J = 5-4$) transition and covering a frequency width of 320 MHz (950 km s^{−1} velocity range at 101 GHz). The quasar 3C273 was used for both reference and bandpass calibrations. Flux calibration was done by observing Mars and/or Uranus, and the uncertainty in the absolute flux is $\sim 20\%$.

During the first observing period (November 1994 and February 1995), we obtained nearly 35 hours of on-source integration using the D- (the most compact) and the C- (the second compact) Array configurations and achieved a typical r.m.s. noise of 3.4 mJy for a binning of 150 km s^{−1}. In the second period (November and December 1995), we had nearly 35 hours of on-source integration with the D-Array configuration and achieved an r.m.s. noise of 2.5 mJy.

The data of the first observing period showed an emission-line feature with 2.2 σ significance at ~ 101.19 GHz ($z = 4.695$).

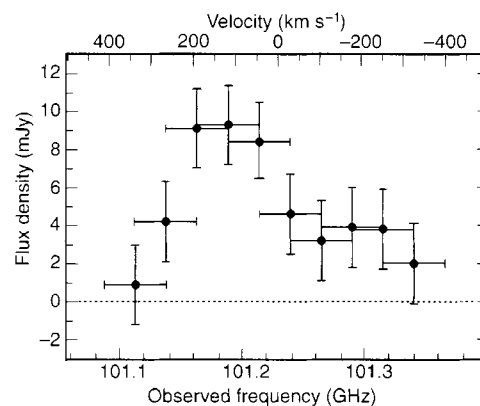


FIG. 1 Spectrum of the CO ($J = 5-4$) emission line, smoothed at a step size of 74 km s^{−1} with a 148 km s^{−1} velocity bin. The velocity scale refers to 101.227 GHz. Horizontal bars show the velocity bins and vertical bars represent the typical r.m.s. noise per beam (2.1 mJy). The dust continuum emission at $\nu_{\text{obs}} = 101$ GHz, estimated by extrapolating the results of Isaak *et al.*¹⁷, is < 1 mJy, thus most of the observed flux density comes from the line emission. On the higher-frequency side of the peak, a tail or another peak is detected with $\sim 2\sigma$ significance over the three velocity bins at around 101.28 GHz; its existence, though, needs further confirmation.

The position of the signal corresponded to the optical position of the quasar within the uncertainty of the CO observations (~ 5 arcsec in the north–south direction and ~ 2 arcsec in the east–west direction). In the second run, we detected again similar feature with 6.2 σ at 101.19 GHz, thanks to a lower system noise during this period. The position, peak frequency, peak flux and velocity width of the feature detected in these two runs all agreed well with each other. We checked the bandpass characteristics using the data for 3C273 and found no feature similar to the emission profiles. The source was not resolved spatially in either of the runs. Finally, we combined the data of both periods with a 1:2 weighting to take account of the difference in the r.m.s. noise. Table 1 summarizes the results.

Figure 1 shows the resultant profile of the averaged flux density of the feature. It is clear that there exists a significant signal (4.4 σ) at around $\nu_{\text{obs}} = 101.19$ GHz and that this is a line emission because the flux densities drop at both sides of the peak. Figure 2 shows the map after processing with the CLEAN algorithm. The signal at the centre of the field is conspicuous (6.6 σ) with a peak flux density of 7.3 mJy. The total flux of this component is calculated to be 2.7 ± 0.41 Jy km s^{−1} neglecting the contribution of the dust continuum emission.

We conclude, after careful consideration of possible contam-

TABLE 1 Summary of the observational results

	Nov. 1994, Feb. 1995	Nov., Dec. 1995	Combined data*	Units
ν_{obs} (peak)	101.19 ± 0.03	101.19 ± 0.03	101.19 ± 0.03	GHz
Z_{peak}	4.695 ± 0.002	4.695 ± 0.002	4.695 ± 0.002	
$\Delta \nu$ (FWHM) [†]	180 ± 74	220 ± 74	220 ± 74	km s ^{−1}
S_ν (peak) [‡]	8.6 ± 3.4	11.5 ± 2.5	9.3 ± 2.1	mJy
$\Sigma S_\nu \Delta \nu$ [§]	1.6 ± 0.74	3.2 ± 0.52	2.7 ± 0.41	Jy km s ^{−1}

* Data from both periods combined with a weight of 1:2 determined from the r.m.s. noise value in each period.

[†] The uncertainties of velocity width show the sampling step size used in obtaining the line profile (Fig. 1).

[‡] The peak flux density when smoothed with 150 km s^{−1} bin. The errors indicate the r.m.s. noise per beam.

[§] The values are calculated with velocity width of 370 km s^{−1}, which gives the maximum signal-to-noise ratio.

ination, that the significant peak must be the ^{12}CO ($J = 5-4$) transition line redshifted from the $z = 4.695$ object. No likely emission line from a Galactic object or the damped Ly α system at $z = 4.38$ or the Lyman-limit system at $z = 4.52$ (ref. 18) on the line of sight to BR1202 – 0725 is expected at the frequency, and the chance probability that another transition line from an object at different redshift comes into the observed narrow frequency band is very low. The redshift of the peak of the CO line is a little larger than that of the Ly α emission¹⁷ line by $\Delta z = 0.006$, which corresponds to a $\sim 300 \text{ km s}^{-1}$ velocity difference at $z = 4.69$, but the value is close to that determined from the Ly α edge¹⁸. Recent observations revealed the presence of Ly α companion at $z = 4.7$ located 2–3 arcsec northwest of the quasar^{19,20}. There is a possibility that the CO emission (or part of it) comes from this companion, though the reported redshift of the companion²⁰ is not exactly the same as that obtained here.

The CO luminosity is often expressed²¹ in units of $\text{K km s}^{-1} \text{ pc}^2$ as

$$L'_{\text{CO}} = (c^2/2k)d_L^2 S_\nu \Delta\nu / [v_{\text{rest}}^2 (1+z)] \\ = 1.8 \times 10^{10} h^{-2} \text{ K km s}^{-1} \text{ pc}^2 \left[\frac{S_\nu \Delta\nu}{2.7 \text{ Jy km s}^{-1}} \right]$$

where d_L is the luminosity distance, k is the Boltzmann constant, S_ν is the observed flux density, $\Delta\nu$ is the velocity width of the emission line and v_{rest} is the rest frequency of the emission line. We use $q_0 = 0.5$ and $H_0 = 100h \text{ km s}^{-1} \text{ Mpc}^{-1}$. The $\text{CO}(J = 5-4)$ luminosity of BR1202 – 0725 is $\sim 1,000$ times larger than that of our Galaxy²², while it is comparable to $L'_{\text{CO}}(J = 4-3)$ and $L'_{\text{CO}}(J = 6-5)$ of F10214 + 4724^{17,23}, and $\sim 1/10$ of $L'_{\text{CO}}(J = 5-4)$ of the ‘Cloverleaf’¹⁴; the latter two are gravitationally lensed objects^{14,24}. We cannot exclude the possibility that the CO emission from the BR1202 – 0725 is also amplified by gravitational lensing. However, an inspection of the Hubble Space Telescope image of the object field does not show any arc-like features¹⁹ and there is no reported evidence to date for lensing for this object. Thus here we derive all the quantities assuming no gravitational-lensing amplification. The far-infrared luminosity of BR1202 – 0725 is estimated from submillimetre-wavelength observations¹⁷ to be $L_{100\mu\text{m}} \approx 6 \times 10^{12} h^{-2} L_\odot (f_{570\mu\text{m}}/100 \text{ mJy})$, where $f_{570\mu\text{m}}$ refers to the observed flux density at $570 \mu\text{m}$. The resultant $L_{100\mu\text{m}}/L'_{\text{CO}}$ ratio, $\sim 400 L_\odot (\text{K km s}^{-1} \text{ pc}^2)^{-1}$, is larger than those of nearby ultra-luminous far-infrared galaxies, $\sim 85 L_\odot (\text{K km s}^{-1} \text{ pc}^2)^{-1}$, and comparable to that of F10214 + 4727, $\sim 200 L_\odot (\text{K km s}^{-1} \text{ pc}^2)^{-1}$, obtained from CO ($J = 1-0$) or ($J = 3-2$) transition lines¹¹.

From the obtained CO luminosity, we estimate the molecular hydrogen mass as

$$M(\text{H}_2) = \alpha L'_{\text{CO}} \\ = 8.3 \times 10^{10} h^{-2} M_\odot \left[\frac{\alpha}{4.5 M_\odot (\text{K km s}^{-1} \text{ pc}^2)^{-1}} \right] \left[\frac{S_\nu \Delta\nu}{2.7 \text{ Jy km s}^{-1}} \right]$$

Because the physical conditions of the gas in a galaxy at $z = 4.7$ are little known yet, we have to be careful in estimating the molecular hydrogen mass by applying the CO-to- H_2 conversion factor, α . The effects of temperature and density may cancel out to some extent¹¹, but the factor may be larger than the adopted value when the metallicity is lower than solar²⁵ as is inferred for such high-redshift objects. Therefore, the true molecular gas mass must be at least of the order of $10^{11} M_\odot$, which is comparable to the stellar mass of a present-day luminous galaxy. Such a large amount of molecular gas at such a high redshift strongly suggests that the galaxy is still in its major star-forming phase.

We now consider the size and average gas mass density of the CO line-emitting region. From the beam size (6 arcsec along the minor axis of the beam pattern) we obtain an upper limit to the size of the CO source of $9 h^{-1} \text{ kpc}$ radius for a spherical object, and a lower limit of the gas mass density of $\sim 3 \times 10^{-2} h M_\odot \text{ pc}^{-3}$, which is already comparable to the mass densities estimated for the cores

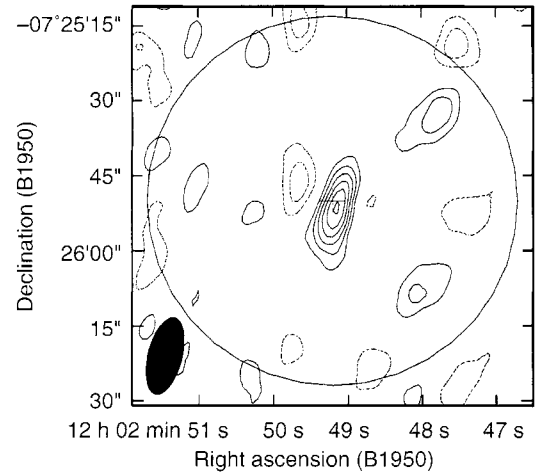


FIG. 2 CO ($J = 5-4$) contour map integrated over the frequency range 101.128–101.253 GHz (370 km s^{-1} velocity bin) constructed by combining all the good data obtained in both observing periods. The cross mark at the centre shows the position of the quasar BR1202 – 0725; right ascension 12 h 02 min 49.2 s, declination $-07^\circ 25' 50''$ (1950.0) (ref. 16). The contours of -2.5 (dashed line), -1.5 (dashed line), 1.5 , 2.5 , 3.5 , 4.5 , 5.5 , and 6.5σ levels are shown, where 1σ corresponds to $0.41 \text{ Jy km s}^{-1}$. The field of view is shown as a circle with a diameter of 74 arcsec , and the synthesized beam pattern ($14 \times 6 \text{ arcsec}$, position angle $\sim 168^\circ$) is also shown as a filled ellipse in the bottom-left corner. The source is not resolved. We estimate the true size of the CO source, assuming that it radiates as a black body, from the relation $T_{\text{b,obs}}(1+z)(\Omega_{\text{beam}}/\Omega_{\text{source}}) + T_{\text{CMB}}(1+z) = T_{\text{ex}} \approx 80 \text{ K}$, where Ω_{beam} and Ω_{source} are the beam size and the solid angle of the source, respectively, and T_{ex} is an excitation temperature of the gas. $T_{\text{b,obs}}$ is the observed brightness temperature from the observed flux density. $T_{\text{CMB}}(1+z)$ represents the contribution of the cosmic microwave background radiation. A T_{ex} of 80 K is required to emit a CO($J = 5-4$) line and is consistent with the dust temperature. The resultant $\Omega_{\text{beam}}/\Omega_{\text{source}}$ is ~ 900 .

of the dark halos of luminous spiral galaxies today²⁶. The true size of the CO source is estimated as $\Omega_{\text{beam}}/\Omega_{\text{source}} \approx 900$, where Ω_{beam} and Ω_{source} are the beam size and the solid angle of the source, respectively. This means that the filling factor of the source in the beam size is very small. Assuming a single homogeneous source, which gives the minimum size of the system, the radius is estimated to be $\sim 500 h^{-1} \text{ pc}$; the size is as large as the cores of elliptical galaxies, implying that we are indeed observing a galaxy-scale object. If the size of the CO source is thus constrained, the average gas mass density is $\sim 20 M_\odot \text{ pc}^{-3} [M(\text{H}_2)/10^{11} M_\odot] [(R/1 \text{ kpc})^{-3}]$, which is comparable to (or somewhat smaller than) the stellar mass densities of the central regions of giant elliptical galaxies or bulges^{27,28} in the present-day Universe. We suggest that this may be evidence for the dissipational collapse of the gas at $z = 4.7$ in this galaxy²⁸.

If we assume a virial equilibrium of the object, the virial mass is estimated as

$$M_{\text{vir}} = R \Delta v^2 / G = 2 \times 10^{11} M_\odot \left[\frac{R}{10 \text{ kpc}} \right] \left[\frac{\Delta v}{300 \text{ km s}^{-1}} \right]^2$$

Taking $M(\text{H}_2) \approx 1 \times 10^{11} M_\odot$, as inferred from the observed CO luminosity, $M(\text{H}_2)/M_{\text{vir}}$ is nearly 1, if we adopt $R \approx 10 \text{ kpc}$ (the approximate beam size). This indicates that the contribution of the gas mass to the total mass is very large there, and that the gas has already concentrated into the central region of the galaxy by some dissipative processes. If we adopt $R \approx 1 \text{ kpc}$ as estimated above, $M(\text{H}_2)/M_{\text{vir}}$ becomes large than unity; this may indicate that the system is not in dynamical equilibrium, or that the observed CO line does not trace the dynamical state of the galaxy (for example, if the object is a rotating disk with a rotational axis pointing approximately towards us, we must include the effect of

the inclination and cannot use the equation above). Alternatively, the CO luminosity might be amplified by a gravitational lensing.

We have found a massive galaxy with a molecular-gas mass comparable to the stellar mass of a present-day luminous galaxy at the Universe age of ~ 1 Gyr. The presence of such a huge amount of metal-enriched molecular gas suggests that the object is in its major star-forming phase, and that star formation in this galaxy started before $z = 4.7$. $\square$

Received 2 January; accepted 4 June 1996.

1. Brown, R. L. & Vanden Bout, P. A. *Astrophys. J.* **412**, L21–L24 (1993).
2. Ohta, K. et al. *Publ. astr. Soc. Japan* **46**, L163–L166 (1994).
3. Wiklund, T. & Combes, F. *Astr. Astrophys.* **288**, L41–L44 (1994).
4. Frayer, D. T., Brown, R. L. & Vanden Bout, P. A. *Astrophys. J.* **433**, L5–L8 (1994).
5. Yamada, T. et al. *Astrophys. J.* **438**, L5–L7 (1995).
6. Ohta, K. et al. *Publ. astr. Soc. Japan* **47**, 739–743 (1995).
7. Yamada, T., Ohta, K., Tomita, A. & Takata, T. *Astr. J.* **110**, 1564–1572 (1995).
8. Evans, A. S. et al. *Astrophys. J.* **457**, 658–670 (1996).
9. Brown, R. L. & Vanden Bout, P. A. *Astr. J.* **102**, 1956–1959 (1991).
10. Kawabe, R., Sakamoto, K., Ishizuki, S. & Ishiguro, M. *Astrophys. J.* **397**, L23–L26 (1992).
11. Solomon, P. M., Downes, D. & Radford, S. J. E. *Astrophys. J.* **398**, L29–L32 (1992).
12. Tsuboi, M. & Nakai, N. *Publ. astr. Soc. Japan* **46**, L179–L182 (1994).
13. Barvainis, R., Tacconi, L., Antonucci, R., Alloin, D. & Coleman, P. *Nature* **371**, 586–588 (1994).

14. Barvainis, R. in *CO: Twenty-five Years of Millimeter-wave Spectroscopy* (Int. Astr. Un. Symp. No. 170, Kluwer, Dordrecht, in the press).
15. Irwin, M., McMahon, R. G. & Hazard, C. in *The Space Distribution of Quasars* (ed. Crampton, D.) 117–126 (ASP Conf. Ser. 21, San Francisco, 1991).
16. McMahon, R. G., Omont, A., Bergeron, J., Kreysa, E. & Haslam, C. G. T. *Mon. Not. R. astr. Soc.* **267**, L9–L12 (1994).
17. Isaak, K. G., McMahon, R. G., Hills, R. E. & Withington, S. *Mon. Not. R. astr. Soc.* **269**, L28–L32 (1994).
18. Storrie-Lombardi, L. J., McMahon, R. G., Irwin, M. J. & Hazard, C. *Astrophys. J. Suppl. Ser.* (in the press).
19. Hu, E. M., McMahon, R. G. & Egami, E. *Astrophys. J.* **459**, L53–L55 (1996).
20. Petitjean, P., Pecontal, E., Valls-Gabaud, D. & Chabot, S. *Nature* **380**, 411–413 (1996).
21. Solomon, P. M., Radford, S. J. E. & Downes, D. *Nature* **356**, 318–319 (1992).
22. Wright, E. L. et al. *Astrophys. J.* **381**, 200–209 (1991).
23. Brown, R. L. & Vanden Bout, P. A. *Astrophys. J.* **397**, L19–L22 (1992).
24. Broadhurst, T. & Lehar, J. *Astrophys. J.* **450**, L41–L44 (1995).
25. Arimoto, N., Sofue, Y. & Tsujimoto, T. *Publ. astr. Soc. Japan* **48**, 275–284 (1996).
26. Athanassoulis, E., Bosma, A. & Papaionnou, S. *Astr. Astrophys.* **179**, 23–40 (1987).
27. Silk, J. *Nature* **301**, 574–578 (1983).
28. Kormendy, J. & Sanders, D. B. *Astrophys. J.* **390**, L53–L56 (1992).

ACKNOWLEDGEMENTS. We thank Y. Hagiwara and staff members of the Nobeyama Millimeter Array for their support during our observations, and their continuous efforts to improving the sensitivity of the array. The Nobeyama Radio Observatory is a branch of the National Astronomical Observatory, an inter-university research institute operated by the Ministry of Education, Science, Sports and Culture of Japan. We also thank S. Ikeuchi and N. Arimoto for discussions and continuous encouragement, E. Hu for providing us with information, and B. Vila-Vilaro for reading the manuscript.

CORRESPONDENCE should be addressed to K.O. (e-mail: ohta@kusastro.kyoto-u.ac.jp).

Molecular gas and dust around a radio-quiet quasar at redshift 4.69

Alain Omont*, Patrick Petitjean*†, Stéphane Guilloteau‡, Richard G. McMahon§, P. M. Solomon|| & Emmanuel Pécontal¶

* Institut d'Astrophysique de Paris-CNRS, 98bis Bd Arago, F-75014 Paris, France

† DAEC, URA CNRS 173, Observatoire de Paris-Meudon, F-92195 Meudon, France

‡ Institut de Radio Astronomie Millimétrique, F-38460 St Martin d'Hères, France

§ Institute of Astronomy, Madingley Road, Cambridge CB3 0HA, UK

|| Astronomy Program, State University of New York, Stony Brook, New York 11794–2100, USA

¶ Centre de Recherche Astronomique de Lyon, CNRS UMR 142, 9 Avenue Charles André, F-69561, St Genis Laval, France

GALAXIES are believed to have formed a large proportion of their stars in giant bursts of star formation early in their lives, but when and how this took place are still very uncertain. The presence^{1–6} of large amounts of dust in quasars and radio galaxies at redshifts $z > 4$ shows that some synthesis of heavy elements had already occurred at this time. This implies that molecular gas—the building material of stars—should also be present, as it is in galaxies at lower redshifts ($z \approx 2.5$, refs 7–10). Here we report the detection of emission from dust and carbon monoxide in the radio-quiet quasar BR1202–0725, at redshift $z = 4.69$. Maps of these emissions reveal two objects, separated by a few arc seconds, which could indicate either the presence of a companion to the quasar or gravitational lensing of the quasar itself. Regardless of the precise interpretation of the maps, the detection of carbon monoxide confirms the presence of a large mass of molecular gas in one of the most distant galaxies known, and shows that conditions conducive to huge bursts of star formation existed in the very early Universe.

We used the four-antenna interferometer of the Institut de Radio Astronomie Millimétrique (IRAM) on the Plateau de Bure, France in December 1995 and January 1996, for a total

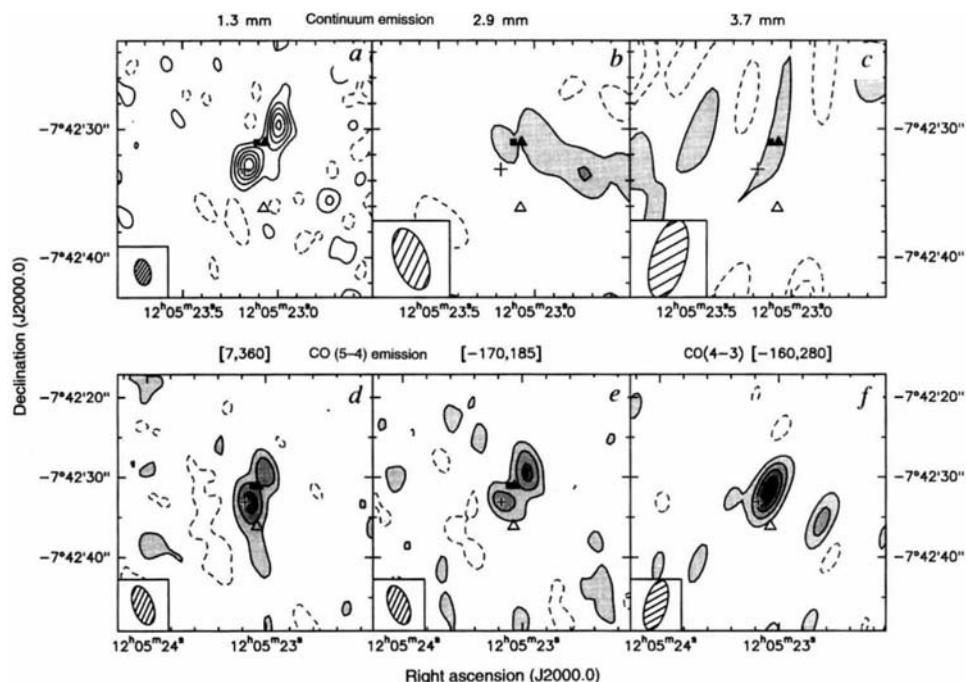
integration time of ~ 16 h with projected baselines from 15 to 160 m. Simultaneous measurements were made at wavelengths λ of 1.35 and 2.9 mm for the purpose of measuring the dust continuum and the CO(5–4) line respectively. The redshift coverage, centred at the approximate redshift of the Ly α line of the companion¹¹, is 4.679–4.730 for the CO(5–4) line (velocity range 2,700 km s^{–1}). The equivalent single-band system temperatures were ~ 120 –150 K and ~ 300 K at 3 and 1.35 mm respectively. Additional data was obtained in April 1996 at $\lambda = 1.35$ mm, and at 3.7 mm to measure the CO(4–3) line over redshift range 4.675–4.709.

The 1.35-mm continuum emission consists of an elongated source at the position of the quasar, and a second component about 4 arcsec to the northwest (NW) with $\sim 35\%$ of the total flux. Figure 1 shows the various features detected in the visible superposed on the millimetre-wave map. The coincidence of the visible position Q of the quasar (marked by a cross) with the main millimetre peak seen on the contour map is almost perfect and well within the uncertainty, ~ 0.5 arcsec, of the relative position of the quasar with respect to the millimetre map frame. The millimetre emission is extended in the NW direction where a companion to the quasar has been detected in the optical and near-infrared broad-band images^{12–14} and confirmed spectroscopically¹¹. However, it is seen in Fig. 1 that there is definitely no coincidence between these companion visible features and the NW 1.35-mm peak.

Spectra in the redshift range 4.679–4.704 of the CO(5–4) line, with a velocity resolution of 60 km s^{–1}, are shown in Fig. 2 at various positions. Line emission is detected towards both sources, with an integrated intensity of 1.1 ± 0.2 Jy km s^{–1} towards the quasar and 1.3 ± 0.3 Jy km s^{–1} towards the NW peak. However, the actual linewidth is uncertain in the latter position. No signal is detected at other positions in the map (Fig. 1d, e and Fig. 2). The displayed fits yield a full-width at half-maximum (FWHM) of 190 km s^{–1} and a central $z = 4.6947$ at Q, and 350 km s^{–1} and $z = 4.6916$ at the NW feature. The derived widths are in the range observed for other high-redshift sources; 220 km s^{–1} for F10214 + 4724⁹, 326 km s^{–1} in H1413 + 117¹⁰, from 150 to 550 km s^{–1} for a sample of 37 ultraluminous galaxies^{7,15}.

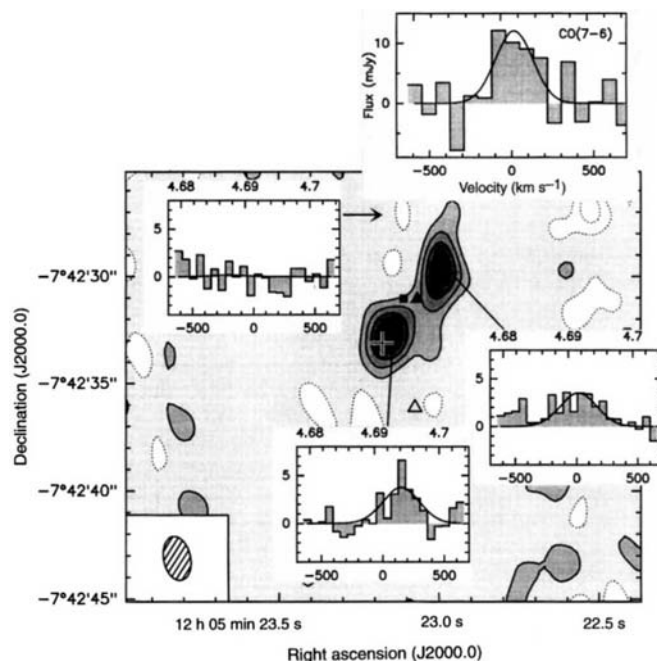
Each of these lines is detected at $\sim (5\text{--}6)\sigma$ level. As both detections are independent because the two positions are spatially resolved, CO(5–4) emission is definitely detected at about the 8σ level. The coincidences with the two 1.35-mm peak positions and the absence of signal elsewhere (Fig. 1d, e) further strengthen this

FIG. 1 1.35-mm and 3-mm maps. For each band, two frequency setups were used to cover a total bandwidth of 0.9 GHz around the redshifted frequencies of the CO(5–4) (101 GHz) and CO(11–10) (223 GHz) lines. Coordinates are in the J2000.0 system. Flux calibration was performed by using 3C273 and 3C279 which are monitored against planets by the IRAM staff; the flux densities were 13–18 Jy (3C273) and 13–16 Jy (3C279) at 223 GHz, with an estimated uncertainty of $\sim 10\%$. Linear polarization of 3C279 has been accounted for in the calibration. The typical phase noise was 10° – 30° at 223 GHz, and twice better at 3 mm. For continuum data, the r.m.s. noise is 0.50 mJy per beam at 223 GHz, 0.22 mJy per beam at 101 GHz, 0.45 mJy per beam at 84 GHz. *a*, 1.35-mm continuum image, obtained using natural weighting. The angular resolution is 2.0×1.3 arcsec, at position angle, PA, of 12° . Contour step is 1.0 mJy per beam or 2σ . Superposed are the positions of the different visible features: Cross, quasar position Q; $\alpha(1950) = 12^h 02^m 49.26 \pm 0.05$ s, $\delta(1950) = -07^\circ 25' 51 \pm 0.5''$ measured from the data obtained with the Cambridge Automated Photographic Measuring (APM) machine. Companion features with respect to the quasar¹⁴: filled triangle, continuum feature; filled square, Ly α peak; open triangle, second continuum feature. The total integrated flux density is 16 ± 2 mJy, and the deconvolved size of the elongated source around Q is $3.0 \times 0.9 \pm 0.7$ arcsec at PA $120 \pm 10^\circ$. Previous continuum measurements with the IRAM 30-m (FWHM 11 arcsec) at 240 GHz are 10.5 ± 1.5 mJy (ref. 1) and 12.3 ± 2.3 mJy (ref. 6). The measurements were, however, performed at different frequencies. Given the very steep dust spectrum, the flux measured with the interferometer at 233 GHz (1.35 mm) should correspond to a flux $\sim 25\%$ smaller than at 240 GHz (1.25 mm), but this should be approximately compensated by the possible small contribution of the CO(11–10) line. Altogether, given the calibration uncertainties of single measurements, especially with the 30-m telescope ($\sim 25\%$), the determinations by the 30-m telescope and the Bure instrument are consistent. *b*, 2.9-mm continuum image. Contour spacings are



0.45 mJy per beam (2σ). Contribution from CO line emission over velocities between -170 and $+360$ km s $^{-1}$ has been avoided. The angular resolution is 5.0×2.5 arcsec at PA 20° . *c*, 3.7-mm continuum. Contour spacings are 0.45 mJy per beam (1σ). Contribution from CO line emission over velocities between -160 and $+280$ km s $^{-1}$ has been avoided. The angular resolution is 6.4×2.7 arcsec at PA 164° . *d*, Map of the CO(5–4) line integrated flux within the velocity range $+7$ to $+360$ km s $^{-1}$, peaking towards the quasar. Contour spacings are 2σ (0.32 Jy per beam km s $^{-1}$, corresponding to 0.9 mJy per beam for continuum emission). Same angular resolution as *b*. *e*, As *c* for the velocity range -170 to $+185$ km s $^{-1}$, peaking towards the NW component. *f*, Map of the CO(4–3) line integrated flux within the velocity range -160 to $+280$ km s $^{-1}$. Contour spacings are 2σ (0.26 Jy per beam km s $^{-1}$, corresponding to 0.6 mJy per beam for continuum emission). Same angular resolution as *c*. The unfavourable beamshape precludes separation of the two components.

FIG. 2 Spectra of the CO(5–4) line emission (histograms in small boxes) superimposed on the 1.35-mm continuum image. Arrows show the location of the emitting region for each spectrum. For each spectrum, the curve is the best-fit gaussian profile with no baseline removed. The large box at top right shows the spectrum of the CO(7–6) line observed with the IRAM 30-m telescope. The velocity and redshift scales are identical for the CO(5–4) and CO(7–6) spectra. The symbols showing the positions of the visible features all the same as in Fig. 1.



conclusion. The lack of pure continuum emission when all the 3-mm broad-band measurements, except in the line frequency range, are included indicates the detected 3-mm emission comes from a spectral line (Fig. 1b).

The presence of CO is further confirmed by the detection of other lines. The 3.7-mm $J = 4-3$ line is detected at the $(5-6)\sigma$ level with integrated flux $1.5 \pm 0.3 \text{ Jy km s}^{-1}$ (see Fig. 1e). No continuum was detected at this frequency (Fig. 1c).

Moreover, the CO(7-6) line (Fig. 2) is present at the 3σ level in a spectrum taken in November 1993 with the IRAM 30-m telescope at Pico Veleta, Spain. Its integrated flux ($3.1 \pm 0.86 \text{ Jy km s}^{-1}$), central redshift (4.6915 ± 0.001) and width ($\sim 250-300 \text{ km s}^{-1}$) are consistent with the 5-4 line detected at Bure, taking into account the large 2-mm beam width of the 30-m telescope (17 arcsec) and excitation conditions similar to F10214 + 4724⁹ and H1413 + 117¹⁶. The observed flux ratio, 3.1/2.4, translates into a brightness temperature (or line luminosity, L') ratio for the (7-6)/(5-4) lines of 0.65, indicating warm gas. To estimate the mass of molecular hydrogen $M(\text{H}_2)$ in BR1202 - 0725, several approximations are required. The high rotational temperature inferred from the above ratio indicates that the line luminosity L' will not be much larger for lower J levels. Thus, using the (5-4) line luminosity and a conversion factor $M(\text{H}_2)/L'_{\text{CO}} = 4$ (see ref. 9), we obtain $M(\text{H}_2) = 6 \times 10^{10} M_\odot$. This is probably an overestimate; the H_2 mass derived this way for ultraluminous infrared galaxies with similar CO luminosities^{7,15} is too high by about a factor of 3.

Another high-redshift object with similar apparent dust and gas properties is F10214 + 4724 at $z = 2.3$, a lensed infrared galaxy. Table 1 details the comparison of the emission from BR1202 - 0725 and F10214 + 4724. It is seen that the observed 3-mm integrated line flux and luminosity as well as the apparent molecular mass, are only a factor ~ 2 smaller in BR1202 - 0725 (OK?). The actual luminosity and H_2 mass depend on an eventual magnification (see below). The relative strengths of the rest-frame far-infrared/submillimetre and CO emissions, after applying a K (redshift) correction for the dust spectrum are strikingly similar in BR1202 - 0725 and F10214 + 4724⁹, and also in H1413 + 117 (the 'Cloverleaf')^{10,17}.

At $z = 4.7$, the ~ 4 arcsec extension seen in millimetre waves typically corresponds to a de-projected distance of $\sim 12-30 \text{ kpc}$. If there is no strong lensing, there are at least two distinct far-infrared emitters with their own heating source (active galactic nuclei, AGN, and/or starburst). The mass of dust, given by equation (1) in ref. 1, should be comparable in both sources and $\sim 10^8 M_\odot$. These two nearby relatively massive galaxies may thus have formed simultaneously, probably in an early stage of the formation of the central core of a cluster, and they could rapidly merge in a time comparable to the timescale of the activity of bright quasars ($\sim 10^8 \text{ yr}$; see, for example, ref. 18). The companion observed in the millimetre continuum could be similar to F10214 + 4724, with most of its energy emitted in the far infrared. However, contrary to F10214 + 4724, it is not detected in the visible. The strong tidal interaction between both objects can boost both the star-formation and AGN activity. It is worth noting that the companion, has a CO redshift $z = 4.6915$, and an FWHM of $250-300 \text{ km s}^{-1}$, very close (with a difference in velocity of

TABLE 1 Properties of BR1202 - 0725 and F10214 + 4724

	BR1202 - 0725		F10214 + 4724		Multiplier	Units
D_L	19		8.8		h^{-1}	Gpc
$S_{1.35\text{mm}}$	16		7.7*			mJy
z	4.69		2.29			
Line	CO(5-4)	CO(7-6)	CO(3-2)	CO(6-5)		
ν_{obs}	101.3	141.2	105.2	210.5		GHz
$S_{\text{CO}} \Delta \nu$	2.4	3.1	4.1 (ref. 9)	9.4 (ref. 9)		Jy km s^{-1}
L'	1.5	1.0	2.6	1.5	$10^{10} A_G^{-1} h^{-2}$	$\text{K km s}^{-1} \text{ pc}^2$
L_{CO}	9	16	3.5	16	$10^7 A_G^{-1} h^{-2}$	$L_\odot$
$M(\text{H}_2)$	0.6		1.0		$10^{13} A_G^{-1} h^{-2}$	$M_\odot$
$X(\text{CO}/1.3)$	2.0		3.2		10^3	km s^{-1}

Symbols used; $h = H_0/100 \text{ km s}^{-1} \text{ Mpc}^{-1}$; A_G is the (eventual) gravitational magnification for millimetre emission; D_L is the luminosity distance (assuming $q_0 = 0.5$); ν_{obs} is the redshifted frequency; $S_{1.35\text{mm}}$ and $S_{\text{CO}} \Delta \nu$ are the measured 1.35-mm flux density and integrated CO flux respectively; L_{CO} and L'_{CO} are the CO line luminosities (equations (1) and (3) of ref. 9); $M(\text{H}_2)$ is the nominal H_2 mass using $M(\text{H}_2) \propto L'_{\text{CO}}$; the ratio $M(\text{H}_2)/L'_{\text{CO}}$ is assumed to be $4 M_\odot (\text{km s}^{-1} \text{ pc}^2)^{-1}$ as in ref. 9 for F10214 + 4724; the ratio $X = (1+z)^{1.5} S_{\text{CO}3\text{mm}} \Delta \nu / S_{1.35\text{mm}}$ should be approximately invariant for the same galaxy moved at different z (see equation (3) of ref. 9 and equation (1) of ref. 1). It is thus a good indication of the relative strength of the far-infrared/submillimetre and CO emission. The difference between BR1202 - 0725 and F10214 + 4724 is hardly significant taking into account the calibration uncertainties and a small correction for the differences of frequency and line excitation. The total flux measured in the (4-3) line, $1.5 \pm 0.3 \text{ Jy km s}^{-1}$, is in good agreement with the (5-4) line.

* $S_{1.25\text{mm}} = 9.6 \text{ mJy}$ from ref. 30, scaled to 1.35 mm.

$\sim 200 \text{ km s}^{-1}$) to that of the metallic absorption system at $z = 4.688^{19}$ (FWHM 260 km s^{-1}). The absorption system may be due to the halo of the millimetre galaxy companion.

The possibility that the double image is due to gravitational lensing should also be considered. Lensing effects are clear on optical images of the other high redshift quasars with CO emission, H1413 + 117 (the 'Cloverleaf', four images²⁰) and F10214 + 4724 (gravitational arc system; see, for example, refs 21, 22 and references therein). Optical amplification factors of ~ 10 or larger are inferred and for F10214 + 4724 the magnification of the CO emission is of the order of 5-10 (ref. 23). The Cloverleaf also clearly shows evidence of lensing in the CO emission²⁴. There are some indications of the possibility of strong lensing on the line of sight to BR1202 - 0725; B. Fort and S. D'Odorico (personal communication) have measured a strong gravitational shear in the field. The line of sight to BR1202 - 0725 itself has strong absorption systems at $z = 1.75$, 2.44 and 4.38 (refs 19, 25, 26, 27). A good explanation of the absence of the second bright optical image could be a strong interstellar absorption in the deflector galaxy along one light path only. Such cases of strong absorption in lens systems are known (see, for example, ref. 28). However, the faint Ly α companion is unlikely to be the result of lensing as it has a spectral energy distribution¹¹⁻¹⁴ and emission line spectrum different from that of the quasar.

In either case (no lensing or modest magnification of the CO emission) these observations clearly show the presence of a large mass of molecular gas and dust in one of the most distant—and hence youngest—quasars. Combined with the other detections of CO in quasars at $z \approx 2.5$ (refs 8, 10), this seems to settle the question of whether high-redshift quasars are associated with galaxies. BR1202 - 0725 has a CO luminosity comparable to the highest observed in nearby luminous infrared galaxies^{7,15}, which show characteristics of huge starbursts and AGNs. Thus, star formation in a copious-metal-enriched interstellar medium existed very early in the history of the Universe, only 0.7 Gyr after the Big Bang ($H_0 = 75$, $q_0 = 0.5$). This supports the idea that the emergence of quasars at very high redshift is connected with the onset of galaxy formation, possibly with formation of the core of elliptical galaxies (see, for example ref. 29), although nearby quasars also show strong CO emission.

The companion of BR1202 - 0725, if real, may be an infrared

galaxy with very high extinction in the optical and ultraviolet. If the companion is representative of young galaxies, massive star formation at very high redshift will be visible primarily at millimetre wavelengths. □

Received 1 April; accepted 4 June 1996.

1. McMahon, R. G., Omont, A., Bergeron, J., Kreysa, E. & Haslam, C. G. T. *Mon. Not. R. astr. Soc.* **267**, L9–L12 (1994).
2. Isaak, K. G., McMahon, R. G., Hills, R. E. & Withington, S. *Mon. Not. R. astr. Soc.* **269**, L28–L32 (1994).
3. Dunlop, J. S., Hughes, D. H., Rawlings, S., Eales, S. & Ward, M. *Nature* **370**, 347–349 (1994).
4. Chini, R. & Krügel, E. *Astr. Astrophys.* **288**, L33–L36 (1994).
5. Ivison, R. J. *Mon. Not. R. astr. Soc.* **275**, L33–L36 (1995).
6. Omont, A. et al. *Astr. Astrophys.* (in the press).
7. Solomon, P. M., Downes, D., Radford, S. J. E. & Barret, J. W. *Astrophys. J.* (in the press).
8. Brown, R. L. & Van den Bout, P. A. *Astrophys. J.* **397**, L19–L22 (1992).
9. Solomon, P. M., Downes, D. & Radford, S. J. E. *Astrophys. J.* **398**, L29–L32 (1992).
10. Barvainis, R., Tacconi, L., Antonucci, R., Alloin, D. & Coleman, P. *Nature* **371**, 586–588 (1994).
11. Petitjean, P., Pécontal, E., Valls-Gabaud, D. & Charlot, S. *Nature* **380**, 411–413 (1996).
12. Djorgovski, S. G. in *Science with VLT* (eds Walsh, J. R. & Danziger, I. J.) 351 (ESO, Springer, Berlin, 1995).
13. Fontana, A., Cristiani, S., D'Odorico, S., Giallongo, E. & Savaglio, S. *Mon. Not. R. astr. Soc.* (in the press).

14. Hu, E. M., McMahon, R. G. & Egami, E. *Astrophys. J.* **459**, L53–L55 (1996).
15. Downes, D., Solomon, P. M. & Radford, S. J. E. *Astrophys. J.* **414**, L13–L16 (1993).
16. Barvainis, R. in *Cold Gas at High Redshift* (eds Bremer, M., Rottgering, H., van der Werf, P. & Carilli, C.) (Kluwer, Dordrecht, in the press).
17. Barvainis, R., Antonucci, R., Hurt, T., Coleman, P. & Reuter, H.-P. *Astrophys. J.* **451**, L9–L12 (1995).
18. Haehnelt, M. J. & Rees, M. J. *Mon. Not. R. astr. Soc.* **263**, 168–178 (1993).
19. Wampler, E. J. et al. *Astr. Astrophys.* (in the press).
20. Magain, P. et al. *Nature* **334**, 325–327 (1988).
21. Broadhurst, T. & Lehar, L. *Astrophys. J.* **450**, L41–L44 (1995).
22. Eisenhardt, P. R. et al. *Astrophys. J.* **461**, 72–83 (1996).
23. Downes, D., Solomon, P. M. & Radford, S. J. E. *Astrophys. J.* **453**, L65–L68 (1995).
24. Scoville, N. Z. et al. in *Cold Gas at High Redshift* (eds Bremer, M., Rottgering, H., van der Werf, P. & Carilli, C.) (Kluwer, Dordrecht, in the press).
25. Storrie-Lombardi, L. J., McMahon, R. G., Irwin, M. J. & Hazard, C. *Astrophys. J.* (in the press).
26. Lu, L., Sargent, W. L. W., Womble, D. S. & Barlow, T. A. *Astrophys. J.* **457**, L1–L4 (1996).
27. Elstou, R., Bechtold, J., Hill, G. J. & Jian, G. *Astrophys. J.* **456**, L13–L16 (1996).
28. Djorgovski, S. G. et al. *Mon. Not. R. astr. Soc.* **257**, 240–244 (1992).
29. Franceschini, A. & Gratton, R. *Mon. Not. R. astr. Soc.* (submitted).
30. Downes, D. et al. *Astrophys. J.* **398**, L25–L27 (1992).

ACKNOWLEDGEMENTS. We are grateful to the IRAM staff at Bure for their assistance. We thank J. Bergeron, S. Charlot, P. Cox, D. Downes, B. Fort, Y. Mellier, S. Radford and P. Schneider for useful discussions. This work was supported by the European Association for Research in Astronomy (EARA).

CORRESPONDENCE should be addressed to A.O. (e-mail: omont@iap.fr).

A simpler approach to Penrose tiling with implications for quasicrystal formation

Paul J. Steinhardt* & Hyeong-Chai Jeong†

* Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

† Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742, USA

QUASICRYSTALS¹ have a quasiperiodic atomic structure with symmetries (such as fivefold) that are forbidden to ordinary crystals^{2,3}. Why do atoms form this complex pattern rather than a regularly repeating crystal? An influential model of quasicrystal structure has been the Penrose tiling⁴, in which two types of tile are laid down according to 'matching rules' that force a fivefold-symmetric quasiperiodic pattern. In physical terms, it has been suggested¹ that atoms form two or more clusters analogous to the tiles, with interactions that mimic the matching rules. Here we show that this complex picture can be simplified. We present proof of the claim⁵ that a quasiperiodic tiling can be forced using only a single type of tile, and furthermore we show that matching rules can be discarded. Instead, maximizing the density of a chosen cluster of tiles suffices to produce a quasiperiodic tiling. If one imagines the tile cluster to represent some energetically preferred atomic cluster, then minimizing the free energy would naturally maximize the cluster density⁶. This provides a simple, physically motivated explanation of why quasicrystals form.

Concerns about the complex atomic interactions required to mimic the original Penrose matching rules have been a prime motivation for alternative models for quasicrystals⁷. Each alternative model treated quasicrystals as some kind of disordered phase that is thermodynamically metastable or stable only at high temperatures. In recent years, through, quasicrystals have been discovered whose diffraction properties, including dynamical scattering effects, indicate near-perfect quasiperiodic order (as perfect as the periodic order exhibited by the best metallic crystals) and whose structure apparently remains thermodynamically stable as temperature decreases⁸.

We first show that a quasiperiodic tiling can be forced using a single type of tile combined with a matching rule; see Fig. 1. The tiling is unconventional (perhaps a better term is a 'covering') as the decagon tiles are permitted to overlap, but only in certain

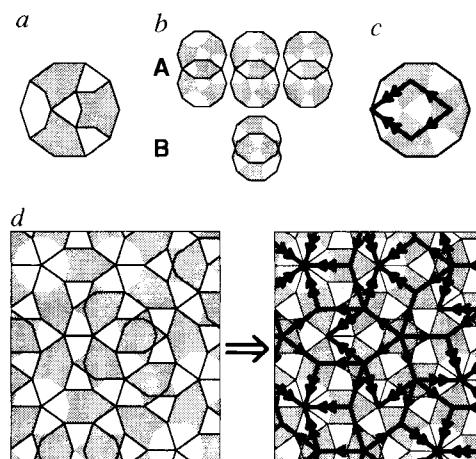


FIG. 1 A quasiperiodic tiling can be forced using a single tile, the marked decagons shown in a. Matching rules demand that two decagons may overlap only if shaded regions overlap and the overlap area is greater than or equal to hexagonal overlap region in A. This permits two possible overlaps between neighbours: either small (A-type) or large (B-type), as shown in b. If each decagon is inscribed with a fat rhombus, as shown in c, a tiling of overlapping decagons (d, left) can be transformed into a Penrose tiling (d, right), where space for the thin rhombi is automatically incorporated.

discrete ways, A- or B-type. As an analogy to a real atomic structure, the overlaps should be construed as the sharing of atoms between neighbouring clusters, rather than interpenetration of two complete clusters. Realistic atomic models of known quasicrystals are known to incorporate clusters whose geometry enables sharing of atoms without distortion of the cluster shape^{7,9–11}.

The decagon construction was originally proposed by Gummelt, who presented an elaborate proof⁵. We offer a very simple, alternative proof which makes clear the relation to Penrose tilings. Our proof is based on inscribing each decagon with a large Penrose 'fat' rhombus tile, as illustrated in Fig. 1c. The original Penrose tiling is constructed from 'fat' and 'thin' rhombi with marked edges such that two edges may join only if the type and direction of arrows match^{2,12}. Gummelt showed that, in a perfect decagon tiling, there are exactly nine ways a decagon can be surrounded by neighbours which have A- or B-type overlaps with it⁵. We have mapped the allowed configurations of overlapping

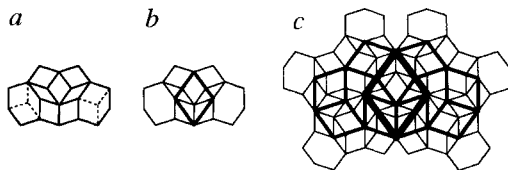


FIG. 2 The cluster C consists of 5 fat and 2 thin rhombi with two side hexagons composed of 2 fat and 1 thin rhombus each. There are two possible configurations for filling each side hexagon; the two possibilities are shown with dashed lines on either side in a. Under deflation, each C-cluster can be replaced by a single 'deflated' fat rhombus, as shown in b. There is a configuration of nine C-clusters shown in c (thin lines) which, under deflation, forms a scaled-up C configuration (medium lines), called a DC-cluster. Under double-deflation, each DC-cluster is replaced by a 'doubly deflated' fat rhombus (thick lines).

decagons into configurations of inscribed rhombi (H.-C.J. and P.J.S., manuscript in preparation). For any two overlapping decagons, the inscribed rhombi share at least one vertex and sometimes share an edge. Where the rhombi join at a vertex only, there is an open angle formed by the edges which are the location and shape where rhombi can be fitted according to the Penrose matching rules. Seven of the nine decagon configurations correspond to completely surrounding a fat tile by neighbouring tiles. In the other two cases, one rhombus vertex is incompletely surrounded; but, there are only two allowed ways of adding overlapping decagons so that the inscribed rhombi complete the vertex. Counting all of these, the decagon overlap rules map into 11 ways of completely surrounding a central fat tile with fat and thin tiles, precisely the number and types allowed by the Penrose arrow rules. Restricting the surroundings of every fat tile to these 11 types is equivalent to enforcing the Penrose arrow rules for fat and thin tiles; and, thus, the proof is completed. An important corollary is that the two-tile Penrose tiling can be reinterpreted in terms of a single, repeating motif, similar to periodic crystal (H.-C.J. and P.J.S., manuscript in preparation).

Next we show that a Penrose tiling can be constructed without imposing matching rules. Instead, the tiling can arise simply by maximizing the density of some chosen tile-cluster, C. The notion is that C represents some low-energy, microscopic cluster of atoms, and that minimizing the energy naturally maximizes the cluster density and forces quasiperiodicity. We first imagine all possible tilings constructed from fat and thin rhombi with no matching rules and show that the Penrose tiling uniquely has the maximum density. (Two tilings are considered equivalent if they differ by patches whose density has zero measure.)

We use the cluster C shown in Fig. 2. This choice is motivated by the fact that the C-clusters in a Penrose tiling and the decagons in a decagon tiling are in one-to-one correspondence. Although they have different shapes, their important similarity is that two neighbouring C-clusters can share tiles in two ways isomorphic to the A- and B-overlaps of decagons. (The hexagon sidings in Fig. 2 are introduced to prevent other kinds of overlaps.) Hence, we know the Penrose tiling has the unique property that every C-cluster has an A- or B-overlap with its neighbours. However, this does not prove that it has the maximum ρ_C , defined as the number of C-clusters per unit area in units where the thin rhombus has area equal to unity.

Our formal proof uses the concept of 'deflation'. 'Deflation' corresponds to replacing each complete C-cluster by a larger, 'deflated' fat rhombus (see Fig. 2). The deflated rhombus has τ times the sidelength and τ^2 times the area of the original, where $\tau = (1 + \sqrt{5})/2$ is the 'Golden Ratio'. Because Penrose tilings are self-similar⁴, the density of deflated fat rhombi equals τ^{-2} times the density of original fat rhombi which equals the density of C-clusters: $\rho_C^0 = 1/(3\tau + 1)$. The deflation operation can be repeated: identify all configurations of deflated rhombi which form a scaled-up version of the C-cluster (we call this configura-

tion a DC-cluster) and replace each with a yet-larger fat tile (see Fig. 2). Due to self-similarity, $\rho_{DC} = \rho_C^0/\tau^2$ for a Penrose tiling. For non-Penrose tilings, deflation corresponds to the same replacement wherever nine fat rhombi form a complete C-cluster, but the deflated tiling is not necessarily similar to the original and may include voids. Our proof is by contradiction: if a tiling existed with $\rho_C > 1/(3\tau + 1)$, then deflating it repeatedly increases the density without bound—an impossibility.

Because the C-clusters can overlap, a reliable scheme for assigning, or at least bounding the area occupied by a given C-cluster, is needed. A useful trick is to decorate each C-cluster as shown in Fig. 3. The kite-shaped region, which has area $3\tau + 2$, will be called the 'core-area' of the C-cluster. Although C-clusters can overlap to some degree, the only possibilities for close overlap are A-overlaps, in which the core-areas meet along an edge; or B-overlaps, in which there is a specific overlap of core-areas (Fig. 3). In a Penrose tiling, these core-areas fill the entire plane without holes. If the core-area of a C-cluster is not overlapped by any neighbouring core-areas, it can be assigned (at least) the entire core-area; for these cases, the C-clusters occupy area $\geq 3\tau + 2$, so they decrease the density relative to the Penrose value $\rho_C = 1/(3\tau + 1)$. Two C-clusters with B-overlaps are assigned area less than $3\tau + 1$ due to the overlapped core-areas. Hence we reach an important conclusion: B-overlaps are the only mechanism for exceeding Penrose density.

To exceed the Penrose density, a tiling must have a greater density of B-overlaps than Penrose tiling. However, this condition is not sufficient. In Penrose tiling, every B-overlap of two C-clusters is surrounded by a DC-cluster (see Figs 2 and 3). In a non-Penrose tiling, a fraction of B-overlaps may not be part of a DC-cluster (that is, one or more of the seven other C-clusters that compose a DC-cluster is not present). In these cases, it is straightforward to show by explicit constructions that one can always identify an area attached to the associated B-overlap which does not belong to the core-area of any C-cluster and is not associated with any other B-overlap (H.-C.J. and P.J.S., manuscript in preparation). This 'extra', unassigned area occupies at least as much area as saved by the B-overlap. Hence a B-overlap which is not part of a DC-cluster does not contribute to increasing the density of C-clusters above the Penrose value.

Suppose there was a tiling with a density of C-clusters greater than the Penrose value. Then, we have just shown that it must have a higher density of DC-clusters than in a Penrose tiling, $R_{DC} > \tau^{-2}$, where R_{DC} is the number of DC-clusters divided by the number of C-clusters. Under deflation and rescaling the area by τ^2 , each DC-cluster becomes a C-cluster of the deflated tiling whose density is $\tau^2 R_{DC} \rho_C$. As $R_{DC} > \tau^{-2}$, the deflated tiling has a density of C-clusters that is strictly greater than the original tiling. Repeating the deflation *ad infinitum* would lead to an impossible tiling with an unbounded density of C-clusters.

A corollary is that if the C-cluster density equals the Penrose value, then $R_{DC} = \tau^{-2}$ (the Penrose tiling value) and the C-cluster density in the deflated tiling must equal the Penrose value. This is useful in proving that the Penrose tiling is the unique tiling with $\rho_C = 1/(3\tau + 1)$. Suppose there was a non-Penrose tiling with the

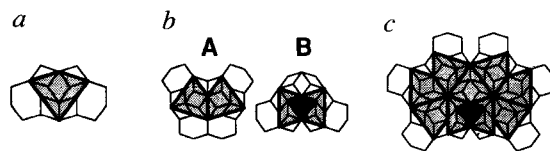


FIG. 3 Associated with each C-cluster is a core-area (with area $3\tau + 2$) consisting of a kite-shaped region, as shaded in a. In a Penrose tiling, core-areas of neighbouring tiles either join edge-to-edge (A-overlap) or overlap by a fixed amount (B-overlap), as shown with dark shading in b. c shows a DC-cluster, illustrating the core-areas of the nine C-clusters which it consists of. An isolated DC-cluster contains one B-overlap (dark shading) surrounded by A-overlaps.

same density. We have argued that the only local configurations that can increase the density above the Penrose value are DC-clusters, and that the increase in density is due to the B-overlap of core-areas, which is the same for each DC-cluster. The corollary is that the hypothetical tiling has the same density of DC-clusters and, hence, the same density of B-overlaps surrounded by DC-clusters as Penrose tiling. But by definition, the non-Penrose tiling must also have patches with non-zero area measure which violate the Penrose matching rules, and so cannot belong to the core-area of any C-cluster. As the DC-cluster density is the same but there are these patches, it would appear that the average area per C-cluster must be less than the Penrose density. The only conceivable exception would be if there happen to be additional B-overlaps which do not belong to DC-clusters whose overlap area exactly compensates the area of the patches. Even this possibility can be eliminated because the corollary states that $R_{DC} = \tau^{-2}$, which means that the density of C-clusters remains unchanged under deflation and rescaling. Yet, the patches grow: a patch excluded from a C-cluster must also be excluded from a DC-cluster, but, also, some C-clusters that border the patches cannot be part of a DC-cluster and add to the patch area (H.-C.J. and P.J.S., manuscript in preparation). As the number of C-clusters remains fixed but the patches grow, the C-cluster density in the deflated tiling must be less than the Penrose value. This contradicts the corollary; hence, uniqueness is established.

The two new approaches to Penrose tiling—a single tile type and maximizing cluster density—can be continued. Together, they suggest that relatively simple criteria can lead to quasicrystal

formation, shedding new light on an old mystery. They suggest that quasicrystals can be understood by considering the energetics of microscopic clusters and that cluster overlap is an important structural element⁶. The concept can be studied using the atom clusters of known quasicrystals. Our two-dimensional tiling results can most readily be applied to decagonal quasicrystals which have periodically spaced layers with Penrose tiling structure. The extension to three-dimensional, icosahedral symmetry is a future challenge. If these principles can be established, they may enable the reliable prediction of new quasicrystals. □

Received 18 March; accepted 11 June 1996.

1. Levine, D. & Steinhardt, P. J. *Phys. Rev. Lett.* **53**, 2477–2480 (1984); *Phys. Rev.* **B34**, 596–616 (1986).
2. Steinhardt, P. J. *Am. Scient.* **74**, 586–597 (1986).
3. Shechtman, D., Blech, I., Gratias, D. & Cahn, J. W. *Phys. Rev. Lett.* **53**, 1951–1954 (1984).
4. Penrose, R. *Bull. Inst. Math. and its Appl.* **10**, 266–269 (1974).
5. Gummelt, P. *Geometriae Dedicata* (in the press).
6. Jeong, H.-C. & Steinhardt, P. J. *Phys. Rev. Lett.* **73**, 1943–1946 (1994).
7. Henley, C. L. in *Quasicrystals, The State of Art* (eds DiVincenzo, D. P. & Steinhardt, P. J.) 429–524 (World Scientific, Singapore, 1991).
8. Goldman, A. et al. *Am. Scient.* **84**, 230–241 (1996).
9. Burkov, S. J. *Phys.* **2**, 695–706 (1992); *Phys. Rev. Lett.* **67**, 614–617 (1991).
10. Steurer, W., Haibach, T., Zhang, B., Kek, S. & Luck, R. *Acta crystallogr.* **B49**, 661–675 (1993).
11. Aragon, J. L., Romeu, D. & Gomez, A. *Phys. Rev.* **B44**, 584–592 (1991).
12. de Bruijn, N. G. K. *Nederl. Akad. Wetensch. Proc.* **A84**, 1–38 (1981).

ACKNOWLEDGEMENTS. We thank F. Gähler for bringing P. Gummelt's work to our attention and for many helpful comments and criticism. We also thank P. Gummelt for sharing her results before publication. This work was supported by the Department of Energy at Univ. Pennsylvania (P.J.S.) and by the USNSF at Univ. Maryland (H.-C.J.).

CORRESPONDENCE should be addressed to P.J.S. (e-mail: steinh@steinhardt.hep.upenn.edu).

Carbon onions as nanoscopic pressure cells for diamond formation

F. Banhart* & P. M. Ajayan†

* Max-Planck-Institut für Metallforschung, Institut für Physik, Heisenbergstrasse 1, 70569 Stuttgart, Germany

† Max-Planck-Institut für Metallforschung, Institut für Werkstoffwissenschaft, Seestrasse 92, 70174 Stuttgart, Germany

SPHERICAL particles of carbon consisting of concentric graphite-like shells ('carbon onions') can be formed by electron irradiation of graphitic carbon materials^{1,2}. Here we report that, when such particles are heated to ~700 °C and irradiated with electrons, their cores can be transformed to diamond. Under these conditions the spacing between layers in the carbon onions decreases from 0.31 in the outer shells (slightly less than the 0.34-nm layer spacing of graphite) to about 0.22 nm in the core, indicating considerable compression towards the particle centres. We find that this compression allows diamond to nucleate—in effect the carbon onions act as nanoscopic pressure cells for diamond formation.

We found recently that electron-irradiation-induced damage to carbon onions is annealed *in situ* at high specimen temperatures³. This enables us to generate and observe onion-like particles with essentially undistorted shells. We used a high-voltage transmission electron microscope (Jeol ARM 1250) operating at 1,250 kV, capable of heating the specimen up to 800 °C. By using a drift compensating system⁴, a point resolution of 0.12 nm was achieved over the whole temperature range. A total pressure of 2×10^{-6} Pa and a hydrogen partial pressure of 6×10^{-7} Pa prevail in the microscope column. Hence, the influence of any gaseous and other impurities can be neglected. Samples containing the well known carbon nanotubes and nanoparticles⁵ were irradiated and imaged at specimen temperatures between 650 and 750 °C. Onions with perfectly undistorted shells formed at these

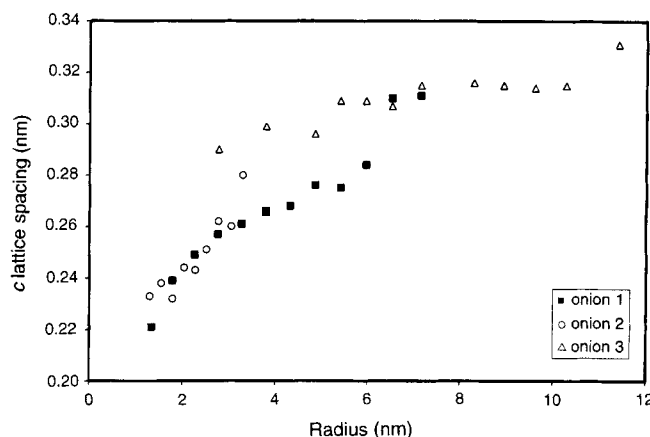


FIG. 1 Distance between the c layers of carbon onions generated at high temperatures as a function of the radius of the shells. Onion 1 (■) has graphitic shells down to the centre (generated at 700 °C). Onion 2 (○) has a hollow core of 2.5 nm diameter (generated at 400 °C). Onion 3 (△) has a diamond core of 4.5 nm diameter (generated at 730 °C).

specimen temperatures under irradiation at high beam intensity (200 A cm⁻²).

In all particles showing straight lattice fringes, such as the nanotubes, the distance between the basal lattice planes is, as expected, close to that of graphite, 0.34 nm. But in the onions, the distance between the lattice planes decreases from outside to inside (Fig. 1). The outermost shells already show a reduced spacing. The lowest value we measured close to the centre was 0.22 nm. After these compressed onion structures are formed, less than one hour of further irradiation at high or reduced beam intensity (20 A cm⁻²) results in the formation of crystallites in the cores of many irradiated onions with more than 15 shells (Fig. 2). The analysis of the lattice images and diffractograms showed that the crystal structure fits precisely to that of cubic diamond. Convergent beam electron diffraction (CBED) with a small electron probe focused onto the diamond cores showed up to

eight reflections of different order that perfectly match to the distance between lattice planes in cubic diamond, and selected-area electron diffraction reveals the most intense rings of cubic diamond (Fig. 3). Energy dispersive X-ray analysis (EDX) with the same small probe showed the carbon $K\alpha$ line as the only relevant peak in the spectrum. We were able to reproduce the transformation of the cores of onions into diamond in a 400-kV microscope, though with less image resolution.

The crystalline regions grow under irradiation; some onions develop a monocrystalline (Fig. 2a), others a polycrystalline core (Fig. 2b) with sizes ranging from 2 to 50 nm. Typical $\langle 111 \rangle$ twins are observed in many of these diamond crystallites. After diamond

crystals are formed in the core, the c lattice spacing of adjacent graphite planes relaxes to slightly higher values (0.25–0.3 nm), compared to onions where no diamond was formed (Fig. 1). On cooling the specimen to room temperature the diamond crystals are found to be stable, whereas the surrounding graphite structure becomes highly defective (Fig. 4), as observed in earlier room-temperature irradiation studies³.

It is apparent that the reduced graphite layer spacing and the nucleation of diamond result from a contraction of the whole irradiated particle. Graphite is very strong in the a – b plane, so we may consider these onions as extremely rigid 'pressure vessels'. We believe that sputtering-induced mass loss from the outer shells

and the dynamics of migration of interstitials are responsible for the contraction of the whole particle³. The outermost two shells of smaller onions already exhibit a reduced spacing (typically 0.28 nm) and as no external pressure can exist, we conclude that already the outermost shell compresses by itself. The nonlinear extrapolation of the measured compression modulus of graphite⁶ shows that, for a contraction of the graphite lattice in the c direction down to a lattice spacing of 0.28 nm, a pressure of 36 GPa is necessary. If we relate this pressure p to the tensile stress σ within the shell by the Poisson formula $p = 2\sigma t/R$ (where t denotes the thickness of the layer, which we assume to be the c lattice spacing in graphite⁷ (0.34 nm), and R is the radius of the shell) we obtain extremely high values for σ ; several hundred gigapascals for outer shells of a few nanometres radius. Also, if we consider the wall of a hollow onion as a continuum, the ratio $2t/R$ being close to unity, we obtain stresses σ of the order of 50–100 GPa within the wall. These estimated values are much higher than the measured tensile strength of graphite whiskers (20 GPa; ref. 8). It may then be reasonably assumed that the graphite planes in such onions are highly corrugated⁹ with some sp^3 bond character between the layers. The pressure prevailing inside the onions may be so high that conditions much above the graphite/diamond equilibrium phase line could exist, and the nucleation of diamond could readily occur. At room temperature, however, the lattice of the irradiated onions is disrupted by many boundary-like defects which drastically

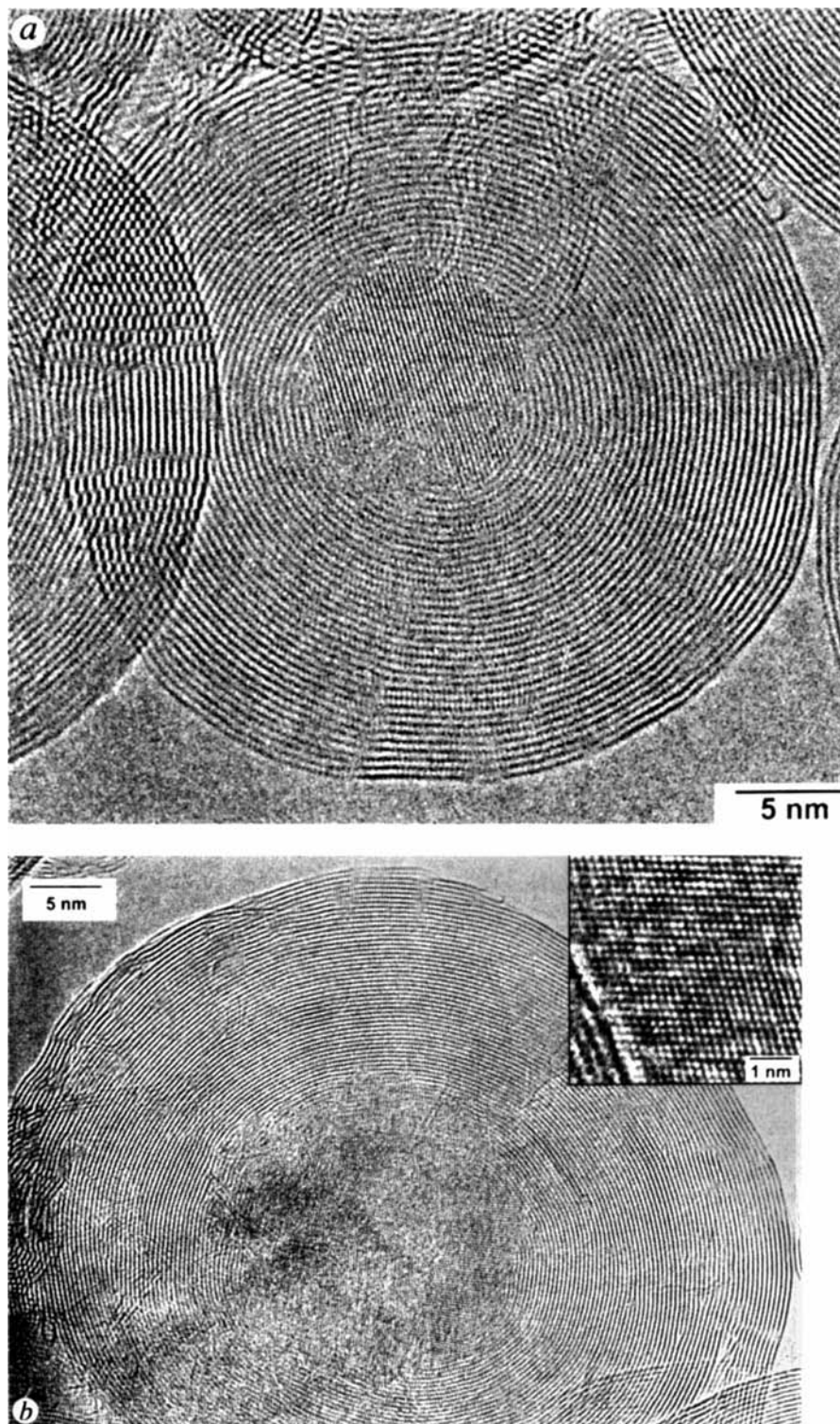


FIG. 2 a, Spherical particle consisting of onion-like shells with a monocrystalline diamond core 10 nm in size. The core shows the $\langle 111 \rangle$ lattice fringes of diamond with a separation of 0.206 nm. The particle was generated under electron irradiation at 730 °C. b, Carbon onion with a polycrystalline diamond core about 20 nm in size, generated during irradiation at 730 °C. Only diamond crystallites with $\langle 111 \rangle$ lattice planes close to the Bragg position (that is, in the right part of the diamond core) can be identified as such. The lattice image of the core corresponds to cubic diamond. Some distortions result from overlapping disordered graphite layers. Inset, CCD image of a section of a monocrystalline diamond core, almost in $\langle 110 \rangle$ projection, which has grown inside a large onion. Part of an overlapping onion is visible in the lower left corner of the inset.

FIG. 3 Selected-area electron diffraction patterns from several carbon onions (a), some of them containing a diamond core, and for comparison from unirradiated source material consisting of polyhedral graphitic particles (b, same scale). The most intense reflection rings of cubic diamond are indexed. The compressed irradiation-induced onions show a broad distribution of graphite lattice spacings, so the graphite rings are considerably smeared. In contrast, the diamond crystals in the cores of the onions exhibit sharp reflections.

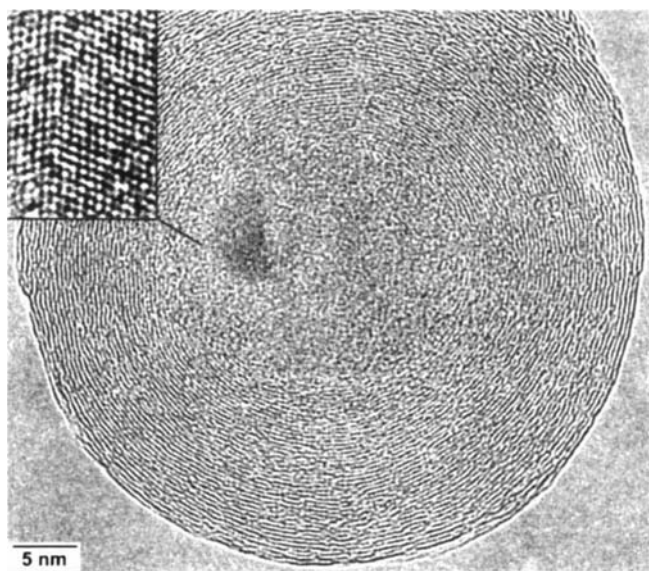
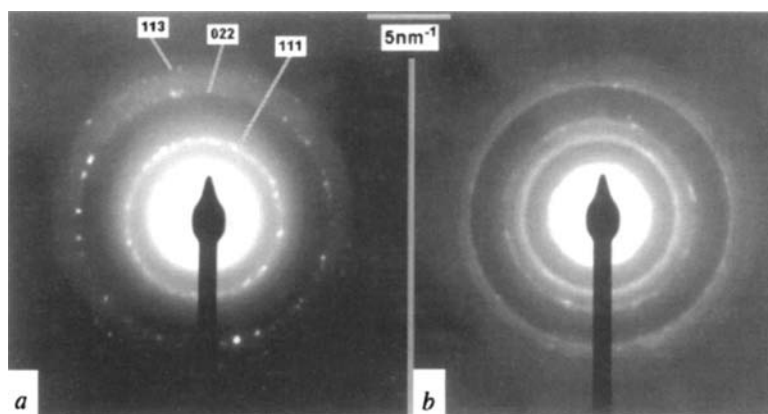


FIG. 4 Onion with a diamond core, formed under irradiation at 700 °C and cooled to room temperature. Irradiation at room temperature has generated a high density of defects in the graphitic shells of the onion. The diamond core, however, remains almost unaffected. A twinned diamond crystallite is seen almost in $\langle 110 \rangle$ projection (shown enlarged in the inset).

reduce the stability of onions and prevent their contraction, thus explaining why formation of diamond does not occur.

In this experiment it is possible to observe the moving boundary between graphite and diamond during growth of the diamond crystals on an atomic scale. In some regions of the cores we observed that a transformation of $\langle 0001 \rangle$ graphite planes to $\langle 111 \rangle$ diamond planes occurs, as has been proposed for the reverse transformation¹⁰. As the densities of (unstrained) graphite and diamond are different ($2,250 \text{ kg m}^{-3}$ compared to $3,510 \text{ kg m}^{-3}$, respectively) the collapse of the centre of the onions during the transformation could lead to a decrease in pressure, resulting in the observed expansion of the graphite lattice around the diamond cores (Fig. 1). In this experiment the irradiation-induced introduction of defects leads to dangling bonds between the layers and may thus promote the corrugation of the graphite planes. The presence of vacancies and interstitials, which are continuously generated during the irradiation, could lower the energy barrier for the transformation of graphite to diamond as proposed by Bar-Yam and Moustakas¹¹.

The transformation of graphite into diamond is of considerable technical interest^{12,13}. Although graphite can be converted to diamond by the application of high temperature and high pressure, the high stability of the basal graphite planes requires that conditions much above the graphite–diamond phase boundary¹⁴, or the presence of catalysts, are necessary for diamond formation. The growth of CVD diamond at low temperature and pressure needs the presence of atomic hydrogen^{13,15}. The conversion of C_{60} to diamond has been achieved at room temperature by applying non-hydrostatic pressure of 20 GPa (ref. 16). We believe that our finding of a new way to convert graphite-like carbon to diamond could lead to new understanding of the nature of the direct graphite–diamond transition. Moreover, carbon onions with compressed shells might show interesting properties such as modified electrical conductivity or a localization of electrical charge. Also, foreign materials inside hollow graphitic particles¹⁷ could be subjected to extreme pressures by applying irradiation at high temperature. □

Received 24 April; accepted 11 June 1996.

1. Kroto, H. W. *Nature* **359**, 670–671 (1992).
2. Ugarte, D. *Nature* **359**, 707–709 (1992).
3. Banhart, F., Ajayan, P. M., Redlich, Ph. & Füller, T. *Phys. Rev. Lett.* (submitted).
4. Höschen, R., Sigle, W. & Philipp, F. in *Proc. 11th European Congress on Microscopy* (in the press).
5. Ebbesen, T. W. & Ajayan, P. M. *Nature* **358**, 220–222 (1992).
6. Lynch, R. W. & Drickamer, H. G. *J. chem. Phys.* **44**, 181–184 (1966).
7. Ruoff, R. S. & Ruoff, A. L. *Nature* **350**, 663–664 (1991).
8. Bacon, R. J. *appl. Phys.* **31**, 283–290 (1960).
9. Sandré, E., Julien, J.-P. & Cyrot-Lackmann, F. *J. Phys. Chem. Solids* **55**, 1261–1268 (1994).
10. De Vita, A., Galli, G., Canning, A. & Car, R. *Nature* **379**, 523–526 (1996).

11. Bar-Yam, Y. & Moustakas, T. D. *Nature* **342**, 786–787 (1989).
12. Clarke, R. & Uher, C. *Adv. Phys.* **33**, 469–566 (1984).
13. Angus, J. C. & Hayman, C. C. *Science* **241**, 913–921 (1988).
14. Bundy, F. P. *Physica A156*, 169–178 (1989).
15. Lambrecht, W. R. L. et al. *Nature* **364**, 607–610 (1993).
16. Núñez Regueiro, M., Monceau, P. & Hodeau, J.-L. *Nature* **355**, 237–239 (1992).
17. Ruoff, R. S., Lorents, D. C., Chan, B., Malhotra, R. & Subramoney, S. *Science* **259**, 346–347 (1993).

ACKNOWLEDGEMENTS. We thank R. Höschen for indispensable technical assistance. P.M.A. acknowledges the Alexander-von-Humboldt-Stiftung for support.

CORRESPONDENCE should be addressed to F.B. (e-mail: banhart@wselix.mpi-stuttgart.mpg.de).

An estimate of global ocean circulation and heat fluxes

Alison M. Macdonald* & Carl Wunsch

Department of Earth, Atmospheric and Planetary Sciences,
Massachusetts Institute of Technology, 77 Massachusetts Avenue,
Cambridge, Massachusetts 02139, USA

ALTHOUGH ocean circulation and the consequent exchange of heat and gases with the atmosphere exert a strong influence on climate, discussions of global circulation have previously been highly schematic¹⁻³ (invoking laminar flow patterns that ignore the turbulent nature of the real flow), non-quantitative and/or based upon mutually inconsistent regional studies¹⁻⁸. Here we present a dynamically and kinematically consistent estimate of the magnitude and structure of global ocean circulation and its associated heat fluxes, derived by integrating hydrographic velocity data over the rapid spatial variations that they show. We find no single overturning cell, but instead a complex and probably time-varying circulation pattern. The simplest interpretation suggests that there are two nearly independent cells: one connecting overturning in the Atlantic Ocean to other basins through the Southern Ocean, and the other connecting the Indian and Pacific basins through the Indonesian archipelago.

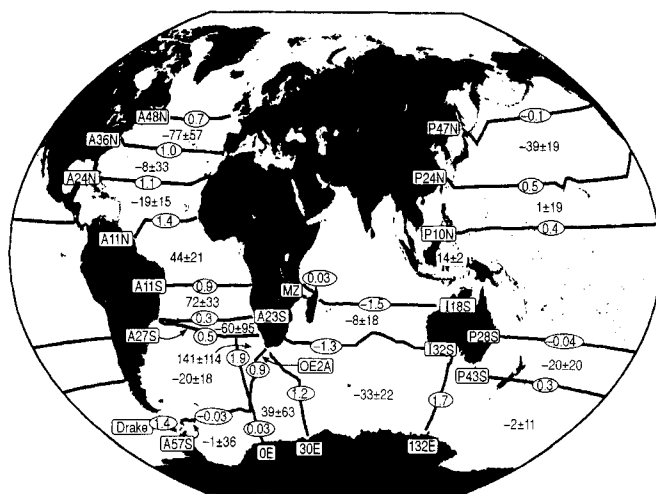
The data (temperature, salinity, oxygen and nutrient measurements along 23 hydrographic sections; Fig. 1) are combined using an inverse box model technique⁹ to estimate the flow field. The model (Box 1) is represented by a set of noisy, under-determined linear simultaneous equations (993 equations in 1,930 unknowns) which are solved using a recursive, tapered weighted least-squares method¹⁰. The system is solved for estimates of the unknowns (geostrophic reference-level velocities and diapycnal fluxes) and their uncertainties. The solution cannot be regarded as definitive—it will change as new data are obtained. Many variations were tried; here we describe only our present best-estimate result. The best-estimate is a combination of statistical guidelines from the inversion with a certain amount of subjective oceanographic opinion.

The global circulation is a complicated turbulent flow whose details are not displayed here. But if the mass and corresponding property fluxes are integrated across ocean basins, a comparatively simple (but still complex) circulation emerges. Figure 2 is a simplified schematic representation of the general circulation for warm and cold temperature ranges and which should be temporally stable in its major elements.

Within the two temperature ranges shown, the integrated Atlantic-overturning cell carries an average of $(16 \pm 5) \times 10^9$ and $(17 \pm 5) \times 10^9$ kg s⁻¹, respectively. Fluid sinks at high latitudes in the North Atlantic, flowing out to the remainder of the ocean at depth. The estimated average magnitude of the Atlantic integrated cell is robust to most assumptions, but it is sensitive, for example, to the flux constraint placed upon the North Brazil current at 11°N, a variable and poorly known quantity¹¹.

Relatively warm waters flow northwards in the eastern South Atlantic and cross over to the western basin south of the Equator. The model produces a strong northward transport of upper-layer waters across the central and eastern portions of the northernmost section in the North Atlantic. Northward flows in the northeastern Atlantic have been suggested by some previous studies^{2,13}, but not by others¹. This feature of the circulation could not be removed even by direct restrictions on the flow. Southward flow of deep waters, although generally seen in the western basins, is not always tightly tied to the western boundary.

Within the southwestern Atlantic, the deep waters from the



BOX 1 Global ocean model

Components of model and constraints.

Mass and salt: conservation in all layers; Ekman convergence/divergence within each area = net top-to-bottom geostrophic outflow/inflow; geostrophic + Ekman flux across each section = net inflow/outflow.

Silica: conservation of silica below euphotic zone and above bottom.

Phosphate: conservation of phosphate and oxygen (1:138) below euphotic zone.

Net flux estimates: freshwater fluxes; from Baumgartner and Reiche²⁴ and Schmitt *et al.*²⁵ with an integration reference point at Bering Strait. Salt fluxes; based on Bering St., transport: $(0.8 \pm 0.6) \times 10^9 \text{ kg s}^{-1}$, $S = 32.5$. Antarctic Circumpolar current; $(142 \pm 5) \times 10^9 \text{ kg s}^{-1}$; North Brazil current; $(26.5 \pm 5) \times 10^9 \text{ kg s}^{-1}$; Florida Straits, $(30.8 \pm 0.5) \times 10^9 \text{ kg s}^{-1}$; Kuroshio, $(26.6 \pm 3.3) \times 10^9 \text{ kg s}^{-1}$; Indonesian Passage, $(10 \pm 10) \times 10^9 \text{ kg s}^{-1}$; Weddell Scotia area, net inflow of $0.1 \pm 0.05 \text{ PW}$; Ekman fluxes, ECMWF winds (Note that the Ekman transport the 10° N section in the Pacific was taken from Wijffels¹⁶.)

Topographic constraints: conservation of mass in the eastern basin of the North Atlantic, and in the deep equatorial Pacific; zero net flux below the sill depth (1) across the Walvis ridge and Agulhas plateau (2) within Mozambique basin and across the Southwest Indian ridge (3) in the Tasman Sea and the Philippine basin.

Weights: all equations are downweighted by their r.m.s. property value; the expected uncertainty of individual layers is $1 \times 10^9 \text{ kg s}^{-1}$; the expected uncertainty of top-to-bottom equations is $2 \times 10^9 \text{ kg s}^{-1}$; top-to-bottom equations are further downweighted by the uncertainty in the Ekman component; the surface layers are further downweighted by the magnitude of the Ekman component based on an estimated area of outcropping.

Model details.

The model is based on the assumptions of hydrostatic and geostrophic balance so that the so-called thermal wind equations apply. An added assumption is that property fluxes (for example, mass and salt) when integrated over trans-oceanic distances are time-independent over the data set duration (25 years), an assumption which is supported by the few available tests^{26,27}. The models can be summarized as follows: (1) flows across the hydrographic sections are carried geostrophically and by the directly wind-driven surface Ekman layers. (2) The geostrophic flow is made up of two spatially varying parts—one of which is known, determined from the density field alone; the other is unknown, the so-called reference level velocity (integration constants) defined to be the geostrophic flow at an arbitrary, specified depth. (3) The Ekman mass fluxes are obtained from operational analysis wind stress values²⁸. (4) Mass and other properties (for example, salt and silicate) are conserved within boxes defined by land, the sea surface and sea floor, the sections and surfaces of constant density up to observational and model error. Note that not all properties are conserved in all boxes. (5) The net fluxes across the sections are constrained with prior estimates and uncertainties. (6) The exchanges between boxes in contact in the vertical (that is, separated by constant density surfaces) are parametrized by a simple uniform flux over the box area. (7) The reference levels are a function of position and are chosen on the basis of prior estimates of depths of estimated weak flow. (8) All constraints are accompanied by an estimate of their uncertainty. The particular constraints used here are similar to those used by Macdonald⁶, and the model is similar to others^{7,9,29,30} used for regional studies. Details of the computations may be found in Macdonald¹⁰.

whether having passed into and possibly through the Indian and Pacific basins, they mainly re-enter the South Atlantic through Drake Passage (the 'cold water' path) or, around the southern tip of South Africa (the 'warm water' path).

The complexity of the circulation indicated between the Atlantic and Indian sectors of the Southern Ocean suggests that simple, steady depictions will not produce viable quantitative estimates of the average westward flow entering the southeastern Atlantic. The model does show evidence, in the South Atlantic east of the Greenwich Meridian, of a strong northward transfer of surface and intermediate waters originating from Drake Passage. There is also ample evidence of water flowing westward to the south of the Cape of Good Hope and this water has similar properties to those found within the Benguela current. Taking the estimated strength of the flow between the Indian and Atlantic basins as typical would imply that the two routes are both important as sources for deep-water formation in the north. However, the uncertainties associated with the westward transport are large, as the 'warm water' flow is carried by a few strong eddy-scale features and this part of our results may well be temporally unstable, the two routes concerned possibly being dominant at different times.

Another controversy has concerned the strength of the Pacific–Indian flow through the Indonesian Passages (T_{PI} henceforth) and its relationship to the fluid returning to the North Atlantic. With no explicit constraints placed on the throughflow, the model produces an acceptable $T_{PI} = (11 \pm 14) \times 10^9 \text{ kg s}^{-1}$. However, the large uncertainty suggests that the model is capable of supporting both larger and smaller throughflows. Experiments using values of 0 and $20 \times 10^9 \text{ kg s}^{-1}$ (a range consistent with recent estimates^{16–18}) as the T_{PI} constraint, have shown this to be true with neither extreme exhibiting any oceanographically unacceptable features.

A region of influence for T_{PI} was determined by finding those regions where significant mass flux changes occurred when T_{PI} was varied. Although effects were found within the Indian basin as far west as Madagascar, changes are not discernible in the Agulhas

current and retroflexion regions. The 'warm water' path for the Atlantic source appears therefore, to be independent of the magnitude of T_{PI} . Effects of changed throughflow do not affect the magnitude nor the properties of the mass flux through the Drake Passage either, so the 'cold water' path is also apparently uncoupled from T_{PI} . The model thus supports the hypothesis that the Pacific–Indian throughflow is not a significant part of a single overall global pathway.

The results are intrinsically complex, but can be interpreted as consistent with two global integrated cells. The first one connects Atlantic overturning to the Southern Ocean where the Antarctic circumpolar current carries lower deep waters to the Indian and Pacific basins where they are converted to upper deep, and intermediate, waters before returning to the Atlantic via Drake Passage. Some waters also re-enter the southeastern Atlantic having been warmed and upwelled in the Agulhas retroflexion region. The second integrated cell connects the Pacific and Indian basins to the north and south of Australia. In this cell, deep waters pass into the Pacific, upwell in the north and return within the Indian basin as intermediate waters after passing through the Indonesian Passages. The two cells are found to be nearly independent, a result permitting a reinterpretation of the Gordon *et al.*⁵ short-circuited warm water path.

Determination of the oceanic heat budget is important because it is a major component of the climate system. The mass conserving circulation estimate described here allows a calculation of the global oceanic heat budget (Figs 1 and 3). Heat fluxes across complete latitudinal circles are obtained at 47° N , 24° N and 30° S . At 30° S , the estimated heat flux of $-0.9 \pm 0.3 \text{ PW}$ is dominated by a large ($>1 \text{ PW}$) poleward temperature flux in the Indian basin and is not significantly affected by the magnitude of the T_{PI} . At 47° N , the net poleward heat flux of $0.6 \pm 0.3 \text{ PW}$ is dominated by the northward transport into polar regions within the Atlantic basin. The model estimate of $1.5 \pm 0.3 \text{ PW}$ calculated at 24° N is lower than, and only just consistent with, the $2 \pm 0.3 \text{ PW}$ of Bryden *et al.*¹⁹. Most of the discrepancy between our value and theirs

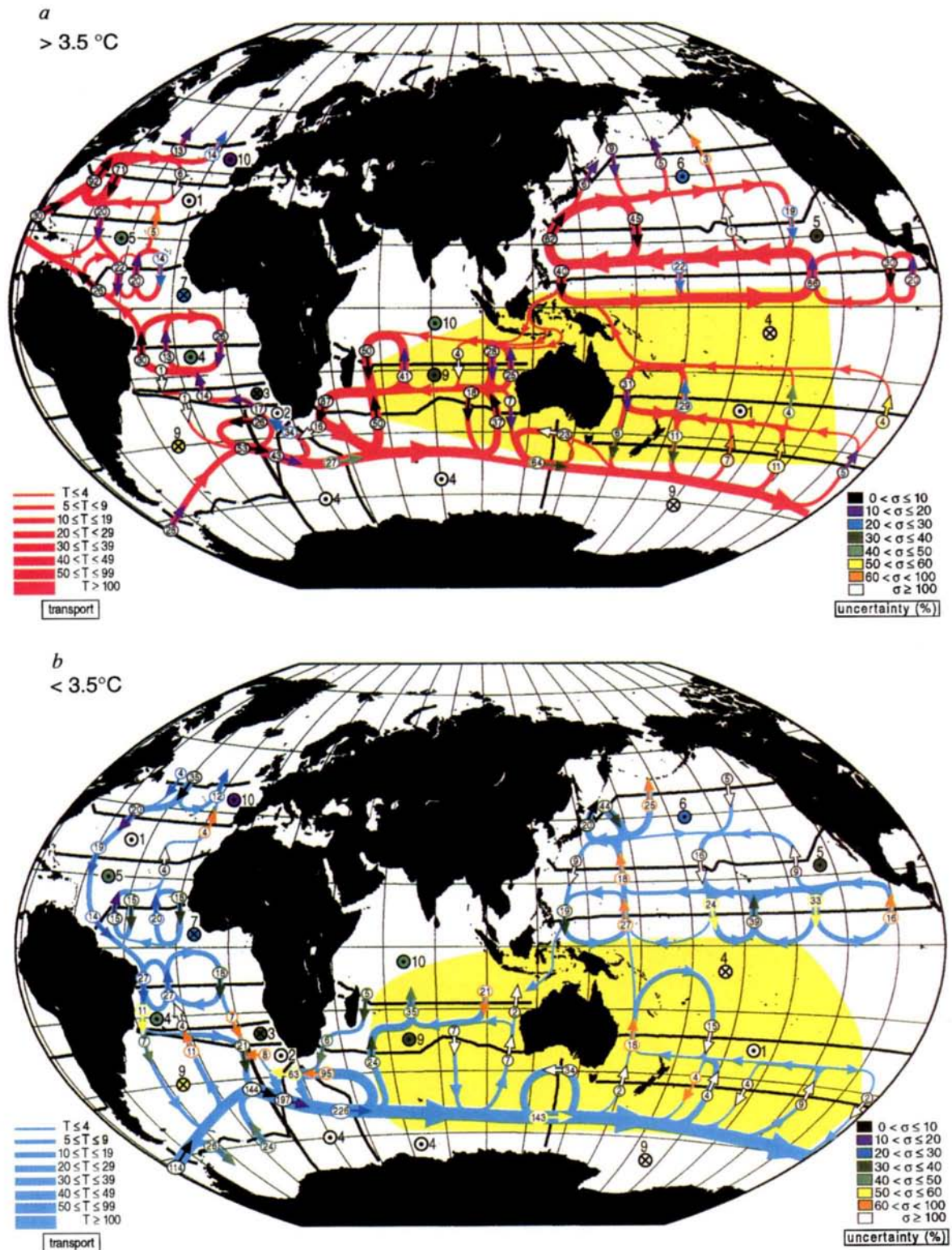
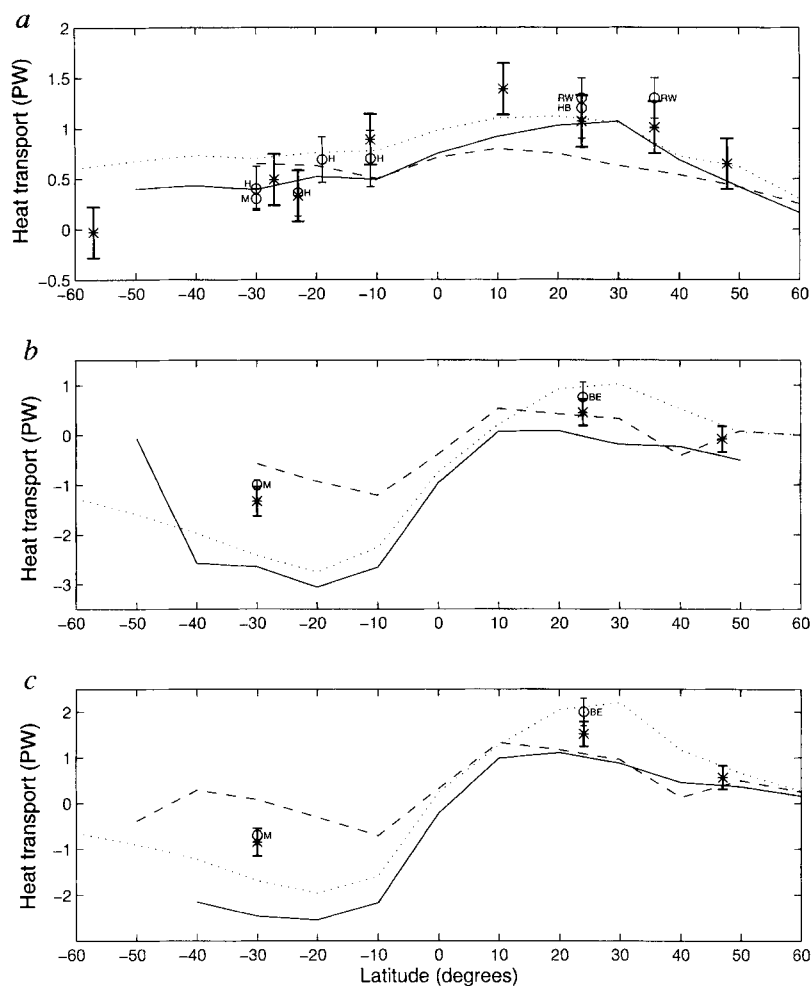


FIG. 2 Schematic representation of the global ocean circulation. Panel a illustrates the flow pattern (in red) of waters of potential temperatures greater than 3.5°C ; panel b illustrates the flow pattern (in blue) for potential temperatures less than 3.5°C . (Potential temperatures are corrected for pressure effects.) The choice of the 3.5°C isotherm is arbitrary. Its depth varies considerably around the globe, lying at about 2,200 m depth in the Atlantic, 1,200 m depth in the Pacific and 1,500 m depth in the Indian Ocean. It outcrops in the northern Atlantic at about 65°N and throughout the Southern Ocean between 50° and 60°S . The strengths of the transports T (in 10^9 kg s^{-1}) are indicated where they cross the sections and are further illustrated by line thickness (see key at left bottom). Colour shading of the vectors indicates, as a percentage, the ratio of the uncertainty in each transport estimate to the estimate itself (see key at right bottom). The vectors are placed geographically midway between

the stations which have been integrated to obtain the transport values, and are not necessarily identifiable with strong local flows. Circles with dots indicate upwelling within a box through the 3.5°C interface. Circles with crosses indicate downwelling. Shading within these symbols indicates the percentage uncertainty (as in the horizontal transport estimates) in the diapycnal transport estimates which are shown next to the symbols. Yellow shading indicates the deduced region of influence of the Pacific–Indian throughflow. Note that the uncertainty of the estimated pathways cannot be calculated from the model results and the temporal instability is likely to be great. The quasi-regularity of the scheme, particularly in the tropical Pacific, results from the very sparse data coverage combined with an attempt to minimize the inferred structure.

FIG. 3 Comparison of best-estimate model meridional heat flux estimates (asterisks with thick error bars) with previously published values (curves and open circles with thin error bars) which were based upon mass-conserving trans-oceanic observations and accompanied by uncertainty estimates. *a*, Atlantic Ocean; *b*, Indo-Pacific Ocean; *c*, All Oceans. Solid curve, Talley³¹ from bulk formulae; dotted curve, Hastenrath³² also from bulk formulae; dashed curve, the numerical model results of Semtner and Chervin³³ which have no error estimates. The reference initials represent the following: BE, Bryden *et al.*¹⁹, HB, Hall and Bryden³⁴; H, Holfort³⁵; M, Macdonald⁶; RW, Rintoul and Wunsch³⁶. All values are in PW. All the computed flux uncertainties are optimistic as they only represent the uncertainty in the absolute transport due to the uncertainty in the reference-level velocities. To roughly account for the difference between an instantaneous value and a long-term average an additional 0.25 PW (ref. 35) was included in the quoted values of uncertainty. Error bars on the previously published values do not include this contribution.



occurs in the Pacific and is due to differing estimates of the Ekman transport there.

The heat flux estimates presented here are robust, but sensitive to the initial estimates of the Ekman transport particularly at 10° N in the Pacific. The pattern of oceanic heat flux divergence (Fig. 1) shows net heat losses in the North Atlantic and Pacific oceans, heat gain around the equatorial regions and loss through-

out much of the Southern Ocean. Southern Ocean divergence estimates are roughly consistent with previous ones^{20,21}. There is strong oceanic heat gain in the small box to the southwest of South Africa—consistent with the findings of Bunker²². This pattern of heat flux divergences becomes a powerful test of atmospheric models²³ and must be regarded as a fundamental component of the present climate system. □

Received 15 January; accepted 5 June 1996.

- Broecker, W. S. *Oceanography* **4**, 79–89 (1991).
- Gordon, A. L. *J. geophys. Res.* **91**, 5037–5046 (1986).
- Schmitz, W. J. Jr. *Rev. Geophys.* **33**, 151–173 (1995).
- Bodden, J. & Schlitzer, R. *J. geophys. Res.* **100**, 15821–15834 (1995).
- Gordon, A. L., Weiss, R. F., Smethie, W. M. & Warner, M. J. *J. geophys. Res.* **97**, 7223–7240 (1992).
- Macdonald, A. M. *J. geophys. Res.* **98**, 6851–6868 (1993).
- Rintoul, S. R. *J. geophys. Res.* **96**, 2675–2692 (1991).
- Schmitz, W. J. Jr. & McCartney, M. S. *Rev. Geophys.* **31**, 29–49 (1993).
- Wunsch, C. *Rev. Geophys. Space Phys.* **16**, 583–620 (1978).
- Macdonald, A. M. thesis, Mass. Inst. of Technol./Woods Hole Oceanogr. Inst. Joint Program, Cambridge, MA (1995).
- Friedrichs, M. A. M. & Hall, M. M. *J. mar. Res.* **51**, 697–736 (1993).
- Martel, F. & Wunsch, C. *J. phys. Oceanogr.* **23**, 898–924 (1993).
- Reid, J. L. *Prog. Oceanogr.* **33**, 1–92 (1994).
- Döös, K. *J. geophys. Res.* **100**, 13499–13514 (1995).
- Garzoli, S. L. & Gordon, A. L. *J. geophys. Res.* **101**, 897–906 (1996).
- Wijffels, S. E. thesis, Mass. Inst. of Technol./Woods Hole Oceanogr. Inst. Joint Program, Woods Hole, MA (1993).
- Fieux, M. *et al.* *Deep-Sea Res.* **41**, 1091–1130 (1994).
- Meyers, G., Bailey, R. J. & Worby, A. P. *Deep-Sea Res.* **42**, 1583–1607 (1995).
- Bryden, H. L., Roemmich, D. H. & Church, J. A. *Deep-Sea Res.* **38**, 297–324 (1991).
- Georgi, D. T. & Toole, J. M. (suppl.) *J. mar. Res.* **40**, 183–197 (1982).
- Guiffreda, M. R. thesis, Texas A&M Univ. (1985).

- Bunker, A. F. *Mon. Weath. Rev.* **116**, 809–823 (1988).
- Foreman, S. J., Alves, J. O. S. & Brooks, N. P. J., *Tech. Rep. 104* (Meteorological Off., Bracknell, UK, 1994).
- Baumgartner, A. & Reichel, E. in *The World Water Balance* (Elsevier Science, New York, 1975).
- Schmitt, R. W., Bogden, P. S. & Dorman, C. E. *J. phys. Oceanogr.* **19**, 1208–1221 (1989).
- Fu, L. *J. phys. Oceanogr.* **11**, 1171–1193 (1981).
- Roemmich, D. & Wunsch, C. *Deep-Sea Res.* **32**, 619–664 (1985).
- Trenberth, K. E., Olson, J. G. & Large, W. G. *NCAR Tech. Note 338+STR* (National Center for Atmospheric Research, Boulder, Colorado, 1989).
- Wunsch, C. *The Ocean Circulation Inverse Problem* (Cambridge Univ. Press, New York, 1996).
- Wunsch, C., Hu, D. & Grant, B. *J. phys. Oceanogr.* **13**, 725–753 (1983).
- Talley, L. J. *J. phys. Oceanogr.* **14**, 231–241 (1984).
- Hastenrath, S. J. *J. phys. Oceanogr.* **12**, 922–927 (1982).
- Semtner, A. J. & Chervin, R. M. *J. geophys. Res.* **97**, 5493–5550 (1992).
- Hall, M. M. & Bryden, H. L. *Deep-Sea Res.* **29**, 339–359 (1982).
- Holfort, J. thesis, Ber. Inst. Meeresk. Kiel (1994).
- Rintoul, S. R. & Wunsch, C. (suppl. 1A) *Deep Sea Res.* **38**, 355–377 (1991).

ACKNOWLEDGEMENTS. We thank J. Toole, J. Marotzke, P. Malanotte-Rizzoli, H. Bryden and W. Schmitz for discussions; D. Spiegel, C. King and C. Gentemann for assistance with programming and data processing; and B. Brown for providing assistance in producing Fig. 2. This work is a contribution to the World Ocean Circulation Experiment and was funded in part by a NASA Global Change Fellowship, NASA and the USNSF.

CORRESPONDENCE should be addressed to A.M.M. (e-mail: alison@oce.orst.edu).

Short magma chamber residence time at an Icelandic volcano inferred from U-series disequilibria

Olgeir Sigmarsson

Centre de Recherches Volcanologiques-CNRS, Université Blaise Pascal, 5, rue Kessler, 63038 Clermont-Ferrand Cedex, France

THE questions of how quickly magmas can differentiate and how long they reside in magma chambers are fundamental to our understanding of magma evolution and the behaviour of volcanoes. Timescales of a few years to a few hundred years have been inferred from physical modelling of crystallization^{1,2}, and from variations in crystal sizes and magma compositions³⁻⁵. Short-lived disequilibria between daughter nuclides of the ²³⁸U decay series yield information about the processes and timescales of magma differentiation^{4,6-14}. Here I report excesses of ²²⁶Ra over ²³⁰Th and ²¹⁰Pb that decrease with differentiation, in lavas from the Vestmannaeyjar volcanic system (Iceland). The decreasing disequilibria can be explained by crystal fractionation of alkali basalt to form hawaiite and mugearite and a 10-year magma chamber residence time. Such rapid differentiation and so short a residence time in a deep reservoir probably result from the injection of a small volume of alkali basalt into a relatively cold crust.

Residence time in magma chambers, defined as the interval between injection and expulsion of a given magma batch, is generally not well constrained. Recycling and remobilization of crystal mush and older non-erupted magmas and their mingling with newly arrived magma may be common in magma chambers of long-lived volcanoes. Residence times are therefore not always easily inferred from isotope data on lavas from mature volcanoes¹²⁻¹⁴.

Table 1 shows results on ²¹⁰Pb–²²⁶Ra–²³⁰Th disequilibria and precise determinations of Rb, Sr, Ba, Pb and Th abundances in lavas from the young Vestmannaeyjar volcanic system¹⁵, in Iceland. Two historic eruptions are well documented from the Vestmannaeyjar archipelago: the 1973 eruption on Heimaey, the largest island, and that of Surtsey (1963–67), which gave birth to a small island 20 km southwest of Heimaey. The Surtsey eruption produced approximately 1.1 km³ of alkali basalts; 0.25 km³ of mugearitic and hawaiitic magma were extruded during the 1973 eruption¹⁵⁻¹⁷. Samples in this study are from a volcanic bomb and a lava flow from Surtsey, a prehistoric basalt formed during the penultimate eruption on Heimaey, and a volcanic bomb and a lava flow from the first day and the final effusive phase of the 1973 Heimaey eruption, respectively. The alkali basalts contain phenocrysts of Cr-spinel (~0.5 volume %), olivine (12–17%) and plagioclase (0–12%), whereas the mugearite from Heimaey contains microphenocrysts of olivine (5%), plagioclase (33%) and titanomagnetite (3%); all the samples are non-cumulative^{15,17}.

All of the Vestmannaeyjar lavas have identical $\delta^{18}\text{O}$, $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{230}\text{Th}/^{232}\text{Th}$ values (5.5‰, 0.7031 and 1.17, respectively) and incompatible trace-element ratios (for example, Th/U), reflecting their origin from a common homogeneous mantle source¹⁸⁻²⁰. Systematic evolution of major and trace elements can be explained by fractional crystallization²⁰. The 1973 hawaiite could be formed by 30% crystallization (15% plagioclase, 8% clinopyroxene, 7% olivine by mass) of the alkali basalt, and the mugearite by 54% crystallization (6% olivine, 27% plagioclase, 10% clinopyroxene, 10% titanomagnetite and 1% hemoilmenite of the hawaiite²⁰. Mass-balance calculations of thorium contents, assuming negligible thorium in minerals, yield exactly the same degrees

of crystallization. During differentiation, the Rb/Th ratio remained nearly constant but Sr/Th decreased because of plagioclase removal. The bulk partition coefficient for Sr (D_{Sr}) was close to unity, in agreement with previous estimates²⁰. This simple differentiation process allows the effects of crystal fractionation on ²²⁶Ra–²³⁰Th and ²¹⁰Pb–²²⁶Ra disequilibria to be assessed from the analysis of only a few samples. The ²³⁰Th decays to ²²⁶Ra, which has a 1,600-yr half-life ($t_{1/2}$) and produces ²²²Rn ($t_{1/2}$ = 3.8 days), which in turn decays to ²¹⁰Pb ($t_{1/2}$ = 22 years) through four very-short-lived nuclides. Disequilibrium in the ²³⁸U decay series can result from a fractionation event between nuclides; however, only a few months after such an event only ²³⁸U–²³⁰Th–²²⁶Ra–²¹⁰Pb remain in measurable disequilibrium. The Vestmannaeyjar lavas have an activity ratio ($^{230}\text{Th}/^{238}\text{U}$) = 1.18 arising from partial mantle melting, and the ²³⁸U–²³⁰Th disequilibrium was not modified during crystal fractionation¹⁹.

The (²²⁶Ra/²³⁰Th) decreases from 1.59 to 1.16 as Th abundance increases from 0.966 p.p.m. in the more primitive basalt from Surtsey to 3.21 p.p.m. in the mugearite (Fig. 1). The 27% decrease in ²²⁶Ra excess during magma evolution is readily explained by incorporation of radium in plagioclase^{4,11,12,14}. The bulk D_{Ra} is estimated (Fig. 1) as 0.25—comparable to values from hawaiites at Etna⁴ and tholeiites at Masaya volcano (O.S., unpublished results). As plagioclase makes up 50% of the crystallizing assemblage, a bulk D_{Ra} of 0.25 corresponds to a D_{Ra} of 0.5 for plagioclase, which is close to recent predictions²¹. Data from Hawaiian basalts²², which lack plagioclase phenocrysts yield a bulk D_{Ra} of 0, implying that only plagioclase removes Ra from basic magmas. The Ba/Th ratio varies irregularly by 20% in Hawaiian basalts (Kilauea and Mauna Loa)²³ and at Vestmannaeyjar, whereas (²²⁶Ra/²³⁰Th) is constant in Hawaii but decreases systematically at Vestmannaeyjar. This implies a different behaviour of Ba and Ra in basic magmas, which is only resolved by high-precision analysis of these two elements.

In contrast to (²²⁶Ra/²³⁰Th), the decay-corrected (²¹⁰Pb/²²⁶Ra)₀ increases from 0.68 to 0.85 with increasing differentiation at Vestmannaeyjar. Due to the short half-life of ²¹⁰Pb, disequilibrium between ²¹⁰Pb and ²²⁶Ra can be detected only in samples that are less than 50–100 years old. In fact, both the prehistoric basalt from

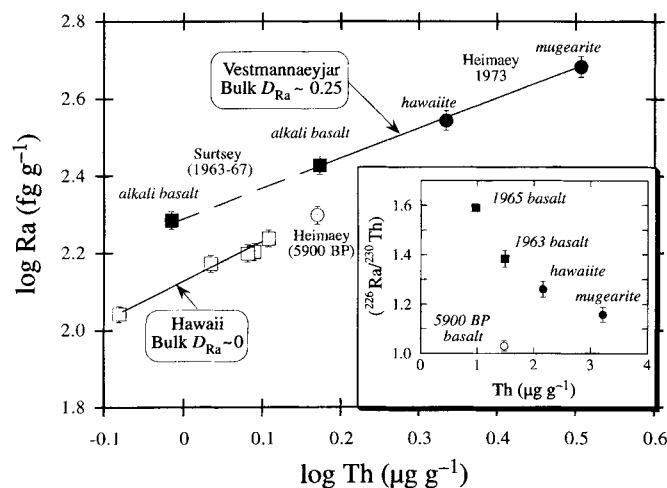


FIG. 1 Variation of radium concentration as a function of thorium during magma differentiation in Vestmannaeyjar. Also shown are data from Kilauea volcano in Hawaii²². The slope on such a log–log diagram is related to the bulk partition coefficient by: $D_{\text{Ra}} = 1 - \text{slope}$, if D_{Th} is 0. The calculated bulk D_{Ra} is 0.25 for the plagioclase-bearing Vestmannaeyjar lavas, but zero for the plagioclase-free Kilauea basalts. Inset, decreasing (²²⁶Ra/²³⁰Th) with increasing Th abundances. The initial (²²⁶Ra/²³⁰Th)₀ value of the prehistoric basalt from Helgafell was probably close to that of the 1963 basalt from Surtsey, which is justified in the light of their identical Th abundances. This allows the age of the penultimate eruption on Heimaey to be calculated as $5,900 \pm 300$ years, which is comparable to a ¹⁴C-age of ~5,500 years attributed to the prehistoric lava flow³².

TABLE 1 Sample descriptions and analytical results

Sample no.	Eruption site	Eruption date	Rock type	Rb (p.p.m.)	Ba (p.p.m.)	Sr (p.p.m.)	Th (p.p.m.)	$(^{230}\text{Th}/^{232}\text{Th}) \pm 2\sigma$	(^{226}Ra) (d.p.m. g $^{-1}$) $\pm 2\sigma$	$(^{210}\text{Pb}) \pm 2\sigma$	$(^{210}\text{Pb}/^{226}\text{Ra}) \pm 2\sigma$	$(^{226}\text{Ra}/^{230}\text{Th}) \pm 2\sigma$	$(^{210}\text{Pb}/^{226}\text{Ra})_0 \pm 2\sigma$	Pb (p.p.m.)	$(^{210}\text{Pb})_0/\text{Pb} \pm 2\sigma$
8756H	Surtsey	2/1965	Alkali basalt	10.1	114	265	0.966	1.17 ± 1	0.440 ± 2	0.51 ± 6	0.87 ± 7	1.59 ± 1	0.68 ± 5	2.04	0.19 ± 2
4638	Surtsey	12/1963	Alkali basalt	14.0	164	362	1.49	1.17 ± 2	0.588 ± 9	0.69 ± 7	0.89 ± 7	1.38 ± 3	0.80 ± 6	2.60	0.24 ± 2
5995	Eldfell	6/1973	Hawaiite	21.2	247	356	2.16	1.16 ± 2	0.77 ± 1	0.97 ± 9	0.92 ± 9	1.16 ± 3	0.85 ± 4	3.68	0.24 ± 1
4694	Eldfell	1/1973	Mugearite	32.6	338	338	3.21	1.17 ± 2	1.06 ± 1	0.96 ± 4	0.92 ± 4	1.04 ± 1	0.96 ± 4	3.73	
VE-67	Heigafell	Prehistoric	Alkali basalt	15.0	197	366	1.48	1.18 ± 2	0.43 ± 6	0.44 ± 7	1.00 ± 7	1.03 ± 2			
LC-1	Lanzarote	9/1730	Basanite				5.75	1.02 ± 1	1.56 ± 3	1.6 ± 2	1.0 ± 1	1.09 ± 2			

Sample 4694-2 is a duplicate. Eruption date is given as month/year. Isotope dilution mass spectrometry was used for the determination of Rb, Ba, Sr and Th with replicate errors of 0.5–1%. Most $(^{230}\text{Th}/^{232}\text{Th})$ ratios were measured by α -spectrometry, and ^{226}Ra , ^{228}Ra and ^{210}Pb by γ -spectrometry in 1993. All samples have $(^{226}\text{Ra}) = (^{228}\text{Th})$. Samples with < 1 p.p.m. Th need important background correction for the determination of ^{210}Pb , this nuclide was thus not measured in sample 8756H. ^{226}Ra and $(^{230}\text{Th}/^{232}\text{Th})$ in 8756H are from a two-stage VG54-30 mass spectrometer as well as ^{228}Ra for VE-67 and LC-1 (γ -ray spectrometry of these samples gave 0.439 ± 9 and 1.59 ± 4 d.p.m. g $^{-1}$, respectively). The associated errors are 2σ from counting statistics on the last significant digit. Reproducibility based on duplicates for both measurement techniques is better than 2% for ^{210}Pb and ^{226}Ra abundances and $(^{230}\text{Th}/^{232}\text{Th})$, $(^{210}\text{Pb}/^{226}\text{Ra})_0$ and $(^{210}\text{Pb})_0/\text{Pb}$ are values calculated at the time of eruption. Pb abundances were measured on an ICP-MS with a reproducibility, derived from duplicates of two samples, better than 1.5%.

Heimaey and the eighteenth-century Lanzarote basanite (Table 1), as well as old lavas from Etna⁴, have $(^{210}\text{Pb}/^{226}\text{Ra})$ indistinguishable from unity. Crystal fractionation, degassing of lead or radon, and radioactive decay could all affect the ^{210}Pb – ^{226}Ra disequilibrium. Plagioclase might preferentially incorporate Pb (refs 24,25), but the constant $(^{226}\text{Ra})/\text{Pb}$ ratio (Fig. 2) indicates that $D_{\text{Ra}} = D_{\text{Pb}}$ for plagioclase in Vestmannaeyjar magmas, and shows that crystal fractionation was not the cause of increasing $(^{210}\text{Pb}/^{226}\text{Ra})$. Degassing of lead and radon is also unlikely. Studies of very-short-lived nuclides (^{210}Pb , ^{210}Bi , ^{210}Po) indicate that lead is enriched by only 1% or less in the gas phase^{26,27}. Furthermore, the two samples from the 1973 Heimaey eruption have identical $(^{210}\text{Pb}/^{226}\text{Ra})_0$ values, even though the mugearite was emitted during the initial explosive phase whereas the hawaiite was extruded quietly as a lava flow six months later. Continuous degassing of ^{222}Rn would interrupt the production of ^{210}Pb from ^{226}Ra in the magma, allowing the ^{210}Pb content to decrease by decay. Thus, a ^{210}Pb deficit in lavas could result from extensive radon loss in a vigorously degassing near-surface magma chamber²⁸, if degassing persisted for several years before eruption. But seismic studies suggest that the magma chamber under Heimaey is more than 15 km deep²⁹; moreover, degassing was detected only during, but not before, the 1973 eruption and bubble rise rates in a mugearitic to hawaiitic magma¹⁷ at 15 km depth would be too slow for ^{222}Rn to escape

before its decay to ^{210}Pb , because of the short ^{222}Rn half-life. Therefore, degassing most probably did not cause, or modify, the ^{210}Pb – ^{226}Ra disequilibrium in the Vestmannaeyjar magmas. The lower $(^{210}\text{Pb}/^{226}\text{Ra})_0$ in the alkali basalt (0.68) and the indistinguishable values in the hawaiite and the mugearite (0.80–0.85) can be explained by fractionation of the basalt to produce the differentiated magmas, and a residence time long enough to allow ^{210}Pb to approach equilibrium with ^{226}Ra .

All of the measured $(^{210}\text{Pb}/^{226}\text{Ra})$ ratios were close to 0.9 in 1993 (Fig. 3), and the different $(^{210}\text{Pb}/^{226}\text{Ra})_0$ values in the 1963 basalt and the 1973 hawaiite and mugearite are consistent with closed-system ingrowth from a common value. This suggests that the residence time of the differentiated magmas was only the 10 years between their eruption and the earlier eruption of their basaltic parent. More generally, the residence time of a closed-system magma chamber can be directly evaluated when the compositions of the parental (influent) and differentiated (effluent) magmas are known. For residence times up to 200 years, ^{226}Ra can be considered as stable as Pb, and the closed-system decay of $(^{210}\text{Pb}/^{226}\text{Ra})$ and $(^{210}\text{Pb})/\text{Pb}$ in differentiated magma are described by:

$$(^{210}\text{Pb}/^{226}\text{Ra}) = [(^{210}\text{Pb}/^{226}\text{Ra})_{t=0} - 1] \times e^{-\lambda t} + 1$$

and

$$(^{210}\text{Pb})/\text{Pb} = (^{210}\text{Pb})_{t=0}/\text{Pb} \times e^{-\lambda t} + (^{226}\text{Ra})/\text{Pb} \times (1 - e^{-\lambda t})$$

where λ is the decay constant of ^{210}Pb , $(^{210}\text{Pb}/^{226}\text{Ra})_{t=0}$ and $(^{210}\text{Pb})_{t=0}/\text{Pb}$ are the values for parental magma entering a

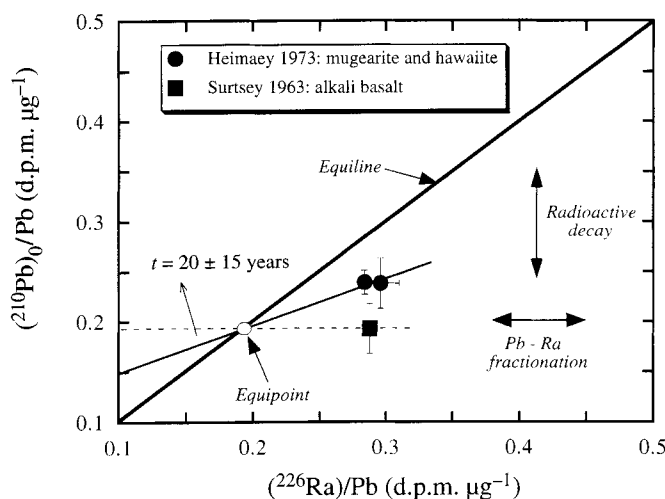


FIG. 2 Isochron diagram (plot of $(^{210}\text{Pb})_0/\text{Pb}$ against $(^{226}\text{Ra})/\text{Pb}$) displaying identical $(^{226}\text{Ra})/\text{Pb}$ in Vestmannaeyjar lavas. Crystal fractionation with 50% plagioclase in the crystallizing assemblage had negligible effect on the $(^{226}\text{Ra})/\text{Pb}$, which strongly indicates similar D_{Pb} and D_{Ra} between the plagioclase and the melt. An 'isochron' is drawn through the 1973 Heimaey lavas and an equipoint, defined by the intersection of the broken horizontal line, representing $(^{210}\text{Pb})_0/\text{Pb}$ of the parental magma, and the equiline on which (^{210}Pb) and (^{210}Ra) are equal. From the slope ($= 1 - e^{-\lambda t}$) of the 'isochron', a residence time of 20 ± 15 years can be estimated for the differentiated magmas. The principles of isochron diagrams are discussed in ref. 33.

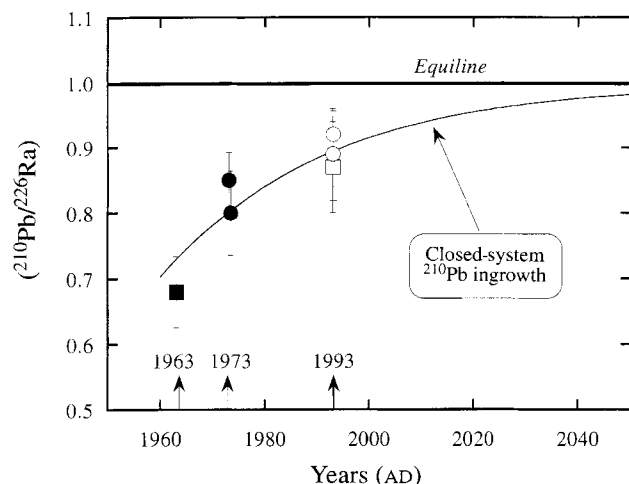


FIG. 3 ^{210}Pb – ^{226}Ra disequilibria as measured in 1993 (open symbols) and decay-corrected to the time of eruption (filled symbols). Circles and squares denote the 1973 differentiated magmas and the 1963 alkali basalt, respectively. All the values are close to the curve of a closed-system ingrowth of ^{210}Pb to equilibrium with ^{226}Ra , indicating that the residence time before the 1973 Heimaey eruption may have been only the 10 years elapsed from the 1963 Surtsey eruption.

magma chamber and t is the residence time. For the 1973 Heimaey magmas, both these expressions give an identical residence time of 20 ± 15 years (Fig. 2).

Combining the ^{238}U -series data presented here with previous results from Vestmannaeyjar leads to the following model for magma evolution in this system. At the time of the alkali basalt eruption at Surtsey in 1963, similar basalts were also emplaced at 15–20 km depth in the crust beneath Heimaey. The crust there was relatively cold, as indicated by its low geothermal gradient³⁰, causing rapid cooling and rapid crystal fractionation of the injected alkali basalt, to form hawaiite and mugearite. The differentiated magma resided in the chamber for about 10 years, during which volatile accumulation led to overpressure in the upper part of the magma chamber and provoked the 1973

Heimaey eruption.

The origin of ^{210}Pb – ^{226}Ra – ^{230}Th disequilibria in Vestmannaeyjar magmas remains unclear, but the decreasing disequilibria with magma differentiation^{4,25,31} are probably inherited from the parental basaltic magmas. An instantaneous radium enrichment during basalt genesis could explain the observed ^{210}Pb – ^{226}Ra – ^{230}Th disequilibria in the 1963 basalt from Surtsey. But this enrichment could be due to partial mantle melting with $D_{\text{Ra}} < D_{\text{Pb}} \approx D_{\text{Th}} < D_{\text{U}}$ only if the basaltic melt erupted immediately after formation. This study shows that magma differentiation effects on radioactive disequilibria must be assessed before ascribing them to partial melting only, and that magma chamber residence time may often be of the order of only a few years or decades. □

Received 28 December 1995; accepted 11 June 1996.

1. Tait, S., Jaupart, C. & Vergnolle, S. *Earth planet. Sci. Lett.* **92**, 107–123 (1989).
2. Brandeis, G. & Jaupart, C. *Contr. Miner. Petrol.* **96**, 24–34 (1992).
3. Mangan, M. T. J. *Volcan. geotherm. Res.* **44**, 295–302 (1990).
4. Condomines, M., Tanguy, J.-C. & Michaud, V. *Earth planet. Sci. Lett.* **132**, 25–41 (1995).
5. Albarède, F. *Geochim. cosmochim. Acta* **57**, 615–621 (1993).
6. Capaldi, G., Cortini, M., Gasparini, P. & Pece, R. *J. geophys. Res.* **81**, 350–358 (1976).
7. Williams, R. W., Gill, J. B. & Bruland, K. W. *Geochim. cosmochim. Acta* **50**, 1249–1259 (1986).
8. Condomines, M. et al. *Nature* **325**, 607–609 (1987).
9. Gill, J. B. & Condomines, M. *Science* **257**, 1368–1376 (1992).
10. Pyle, D. M. *Earth planet. Sci. Lett.* **112**, 61–73 (1992).
11. Reagan, M. K., Volpe, A. M. & Cashman, K. V. *Geochim. cosmochim. Acta* **56**, 1401–1407 (1992).
12. Volpe, A. M. & Hammond, P. E. *Earth planet. Sci. Lett.* **107**, 475–486 (1991).
13. Pyle, D. M., Ivanovich, M. & Sparks, R. S. J. *Nature* **331**, 157–159 (1988).
14. Schaefer, S. J., Sturchio, N. C., Murrell, M. T. & Williams, S. N. *Geochim. cosmochim. Acta* **57**, 1215–1219 (1993).
15. Jakobsson, S. P. *Acta naturalia islandica* **26**, 1–103 (1979).
16. Thorarinnsson, S., Steinthorsson, S., Einarsson, Th., Kristmannsdóttir, H. & Oskarsson, N. *Nature* **241**, 372–375 (1973).
17. Jakobsson, S. P., Pedersen, A. K., Ronsbo, J. G. & Larsen, L. M. *Lithos* **6**, 203–214 (1973).
18. Muehlenbachs, K. & Jakobsson, S. P. *Carnegie Inst. Year Book* **72**, 597–598 (1973).

19. Sigmarsson, O., Condomines, M. & Fourcade, S. *Earth planet. Sci. Lett.* **110**, 149–162 (1992).
20. Furman, T., Frey, F. A. & Park, K.-H. *Contr. Miner. Petrol.* **109**, 19–37 (1991).
21. Blundy, J. & Wood, B. *Nature* **372**, 452–454 (1994).
22. Cohen, A. S. & O'Nions, R. K. *Earth planet. Sci. Lett.* **120**, 169–175 (1993).
23. Hémond, C. et al. *Chem. Geol.* **116**, 163–180 (1994).
24. Reagan, M. (abstr.) *Eos* **69**, 1509 (1988).
25. Rubin, K. et al. *J. Volcan. geotherm. Res.* **38**, 215–226 (1989).
26. Lambert, G., Le Cloarec, M. F., Ardouin, B. & Le Rouille, J. C. *Earth planet. Sci. Lett.* **76**, 185–192 (1985/86).
27. Gill, J. B., Williams, R. W. & Bruland, K. W. *Geophys. Res. Lett.* **12**, 17–20 (1985).
28. Bottinga, Y. & Javoy, M. *Chem. Geol.* **81**, 255–270 (1990).
29. Einarsson, P. *Technophysics* **189**, 261–279 (1991).
30. Flovenz, O. G. & Sæmundsson, K. *Tectonophysics* **225**, 123–138 (1993).
31. Gill, J. B. & Williams, R. W. *Geochim. cosmochim. Acta* **54**, 1427–1442 (1990).
32. Kjartansson, G. *Naturfr.* **36**, 136–145 (1966).
33. Allegre, C. J. & Condomines, M. *Earth planet. Sci. Lett.* **28**, 395–406 (1976).

ACKNOWLEDGMENTS. The Vestmannaeyjar samples were supplied by S. P. Jakobsen from the Icelandic Museum of Natural History. We thank C. Pin for help with the ICP-MS analysis; M. Condomines for discussions; F. Albarède and M. Condomines for comments on an earlier manuscript; and J. Gill for a review. This work was partially financed by the DBT programme of INSU (CNRS) and the Icelandic Science Foundation.

CORRESPONDENCE should be addressed to O.S.

An Early Cretaceous bird from Spain and its implications for the evolution of avian flight

José L. Sanz*, Luis M. Chiappe†, Bernardino P. Pérez-Moreno*, Angela D. Buscalioni*, José J. Moratalla*, Francisco Ortega* & Francisco J. Poyato-Ariza*

*Unidad de Paleontología, Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049-Madrid, Spain
†Department of Ornithology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024, USA

AVIAN flight is one of the most remarkable achievements of vertebrate evolution, yet there is little evidence of its early phases. Specimens of *Archaeopteryx* shed important (albeit controversial) light on this evolutionary phenomenon, but the large morphological (and almost certainly functional) gap between *Archaeopteryx* and modern avians remained virtually empty until recently. Here we report a new, exquisitely preserved, bird from the Lower Cretaceous Konservat-Lagerstätte of Las Hoyas (Cuenca, Spain) which provides evidence for the oldest known alula (bastard wing). Crustacean remains found inside its belly also provide the oldest direct evidence of feeding habits in birds. The new specimen has numerous synapomorphies with the Enantiornithes, but its unique sternal morphology, along with other autapomorphies in the furcula and vertebral centra, support the recognition of a new enantiornithine taxon, *Eoalulavis*

hoyasi. The combination in *Eoalulavis* of a decisive aerodynamic feature, such as the alula, with the basic structures of the modern flight apparatus indicates that as early as 115 million years ago, birds had evolved a sophisticated structural system that enabled them to fly at low speeds and to attain high manoeuvrability.

Fossil birds for the Early Cretaceous of Las Hoyas are among the most informative for understanding the early evolutionary history of birds. Two avian taxa have been described to date: the basal ornithothoracine *Iberomesornis*^{1,2} and the enantiornithine *Concornis*^{3,4}. A new and well-preserved bird provides important evidence about the early stages of the evolution of flight.

Aves L., 1758

Ornithothoraces Chiappe and Calvo, 1994

Enantiornithes Walker, 1981

Eoalulavis hoyasi, gen. et sp. nov.

Etymology. *Eos* (Greek): dawn; *alula* (Latin): bastard wing; and *avis* (Latin): bird; because it provides the oldest evidence of an alula; *hoyasi* (from the Spanish word Hoyas): referring to where it was found.

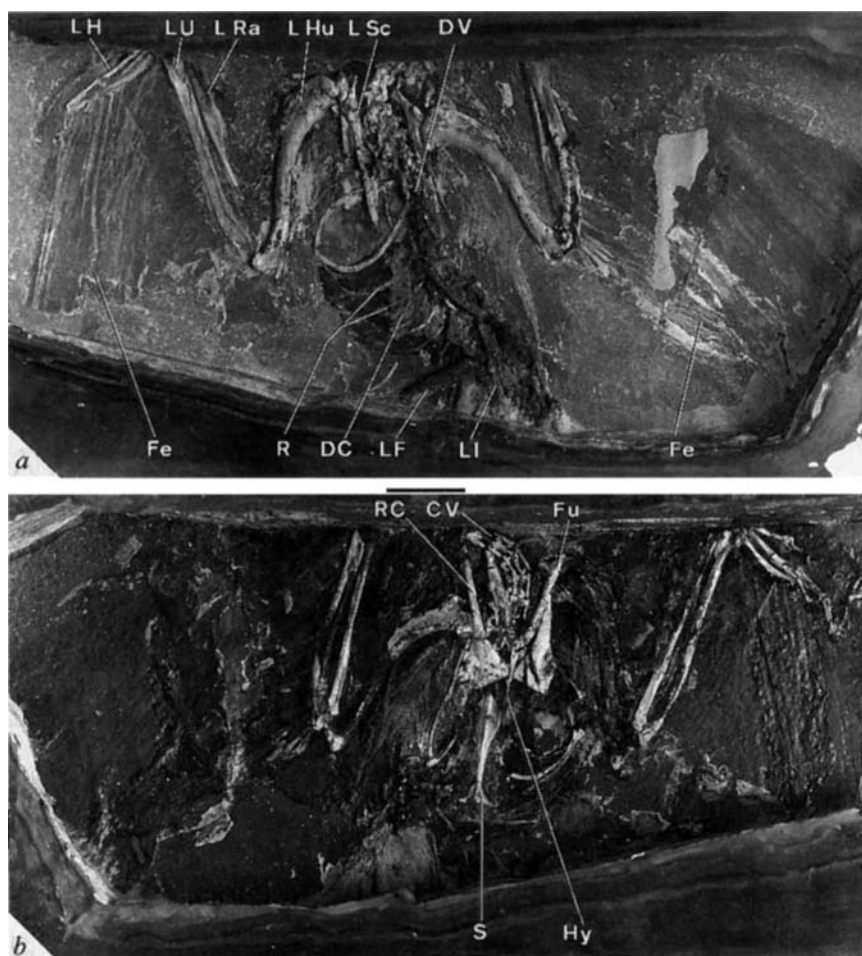
Holotype. See Fig. 1.

Horizon and locality. Las Hoyas fossil site, La Cierva township, province of Cuenca, Spain. Calizas de La Huérguina Formation, Late Barremian (Early Cretaceous)⁵.

Diagnosis. See Fig. 2.

Eoalulavis has a skeletal wingspan of about 17 cm, roughly the size of a goldfinch (*Carduelis carduelis*). The cervical and dorsal centra have deep lateral excavations. Neither uncinat processes nor gastralia are present. The robust furcula has an interclavicular angle of about 45°. Its hypocleidium is extremely long (~77% of the rami) and compressed. The elongated strut-like coracoid has a subtriangular dorsal fossa converging towards a supracoracoid nerve foramen, which opens into a medial groove. Its sternal third

FIG. 1 Holotype specimen (LH 13500 a/b; Las Hoyas Collection, Museo de Cuenca, Cuenca, Spain) of *Eoalulavis hoyasi* (provisionally housed in the Unidad de Paleontología of the Universidad Autónoma de Madrid, Spain) LH 13500 is preserved between a slab and a counterslab. Slabs were prepared with formic acid after embedding them in a frame of transparent polyester resin²³. LH 13500a (dorsal view, a) contains 15 vertebrae (five of them are cervicals), both scapulae, left coracoid and proximal half of the right one, the furcula and its hypocleidium, several ribs, nearly complete left wing and right wing except of the hand, portions of left ilium, proximal end of left femur, and sacrum. The counterslab (LH 13500b, ventral view, b) includes seven vertebrae (five of them are cervicals), both coracoids, proximal end of left scapula, complete furcula and sternum, several ribs, left wing, and humerus and ulna–radius of right wing. Feathers and digestive contents are preserved in both slabs. Although the sternum appears to be only partially ossified, the degree of ossification of the articular surfaces of the remaining elements, as well as the complete ossification of the furcula, which normally ossify late in ontogeny, suggests that this specimen does not represent an early ontogenetic stage. Note that caudal to the ninth preserved vertebra, the specimen is preserved as a mould (copied by the plastic resin) of the diagenetic dissolution of bones. Despite the fact that the bones were accurately moulded, little information can be retrieved from them. Scale bar represents 1 cm. Abbreviations: CV, cervical vertebrae; DC, digestive contents; DV, dorsal vertebrae; Fe, feathers; Fu, furcula; Hy, hypocleidium; LF, left femur; LH, left hand; LHu, left humerus; LI, left ilium; LRa, left radius; LU, left ulna; LSc, left scapula; R, ribs; RC, right coracoid; S, sternum.



is strongly convex laterally, although becoming distally straight. The scapular blade is straight with a sharp end. The well-developed acromion is roughly depressed, somewhat perpendicular to the blade. The morphology of the sternum is unique within dinosaurs (see diagnosis in Fig. 2, and Fig. 3). The lateral margins are smooth and well-defined, lacking either facets for the articulation of the ribs or the coracoids. The humerus has a prominent and cranioventrally projected bicapital area. Its ventral tubercle is caudally projected and separated from the head by the capital incision. Both pneumatic foramen and fossa are absent. The humeral distal end is craniocaudally compressed with transversely oriented condyles. The humerus–ulna length ratio is roughly 88%. The ulnar shaft is twice as wide as the radial one, and the ulna has a weak olecranon and an elongated, strong scar for the bicapital muscle. The radius has a caudal, longitudinal groove and a distinct bicapital tubercle. The hand–ulna length ratio is about 58%. The major metacarpal is shorter than the minor one and is not fused to it distally. The alular and major digits have two and three phalanges, respectively; the distal phalanges are small claws.

Feathers are conspicuous in both slabs (Figs 1 and 4a). Body feathers are visible around the humeri and thoracic girdle. Flight feathers are preserved connected to the distal portion of the wing. Eight primary remiges are preserved, although they are missing in the proximal two thirds of the major metacarpal. Seven secondary remiges attach to the proximal ulnar extremity and one to its distal end. The alular digit has at least one small feather attached to its proximal phalanx. The rachis of this feather is not visible, but its barbs are placed symmetrically. Undoubtedly, this feather corresponds to the alula, or bastard wing.

The skeletal morphology of *Eoalulavis* exhibits a large number

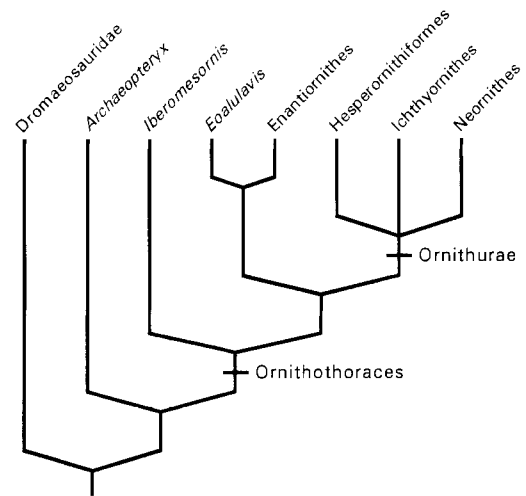
of derived characters used to diagnose the Enantiornithes (Fig. 2), a distinct group of volant ornithothoracine birds distributed world-wide during the Cretaceous^{6,7}. Despite the fact that *Eoalulavis* is easily identifiable as a member of the Enantiornithes, its precise interrelationships must await a comprehensive phylogenetic study of this speciose group (Fig. 2).

At the Las Hoyas Konservat-Lagerstätte, soft tissues are frequently preserved. Examples include both isolated and adhered feathers (such as *Concornis*³), as well as other epidermal remains (like in the ornithomimosaur *Pelecanimimus*⁸ or the albanerpetontid *Celtesaurus*⁹). *Eoalulavis* provides another striking example. In the holotype, in addition to feathers, digestive contents have been found. Scattered organic particles are preserved within a limonitic mass in the thoracic cavity (Fig. 1). Some of these particles are identifiable as crustacean exoskeletal elements, providing the oldest direct evidence of trophic habits in birds (the only other mesozoic case is that of Late Cretaceous *Baptornis*¹⁰). This evidence suggests that *Eoalulavis* had aquatic feeding habits, an interpretation congruent with previous considerations of the Las Hoyas birds as water-related (although not strictly aquatic) inhabitants³.

Specimens with preserved feathers are rare in the fossil record. Even rarer are those in which feathers are arranged in a natural position. In *Eoalulavis*, five and three primaries are attached to the phalanges and the major metacarpal, respectively, and eight secondaries are connected to the ulna. Most remarkable is the preservation of the alula in its natural position (Fig. 4a). This feather constitutes the oldest known alula (it is not preserved in *Concornis* and *Iberomesornis*).

The occurrence of an alula in this early bird sheds significant light on the evolution of avian flight. The alula is essential in

FIG. 2 Phylogenetic analysis. Nelsen consensus cladogram (two most parsimonious trees were obtained) resulted from the analysis of the data matrix, using the implicit enumeration option of the Hennig 86 (ref. 24) program (consistency index, 0.85; retention index, 0.86; length, 100). To establish the relationships of *Eoalulavis* to other basal birds we have scored this taxon in the data-matrix used in ref. 4. We modified this matrix as follows: (1) Dromaeosauridae is the single outgroup; (2) *Patagopteryx* has been excluded from the analysis; (3) *Concornis* has been included within Enantiornithes; (4) Characters 2 (maxillary process of premaxilla forming most of the facial margin) and 5 (osseous external naris considerably larger than antorbital fenestra) in Enantiornithes have been coded as 0 (primitive state), on the basis of new data. *Eoalulavis* and the Enantiornithes are unambiguously united by derived states 72 (prominent bicipital crest of humerus, cranioventrally projected), 74 (convex lateral margin of coracoid), 75 (supracoracoid nerve foramen of coracoid opening into an elongate furrow medially and separated from the medial margin by a thick bony bar), 76 (strong lateral grooves on the bodies of dorsal vertebrae), 77 (parapophyses located in the central part of the bodies of dorsal vertebrae), 83 (lateral face of the humeral bicipital crest with a small fossa for muscular attachment) and 84 (furcula laterally excavated). Derived characters 73 (convex lateral cotyle of ulna, separated from the olecranon by a groove), 78 (caudal projection of the lateral border of the distal end of the femur), 79 (wide and bulbous medial condyle of tibiotarsus), 80 (metatarsal IV significantly smaller than metatarsals II and III) and 81 (trochlea of metatarsal II broader than trochlea of metatarsals III and IV) are ambiguous synapomorphies of these two clades. The position of *Eoalulavis* with respect to the Enantiornithes must not be taken as a sister-group relationship. The present data simply support the placement of *Eoalulavis* on the enantiornithine branch. A comprehensive analysis of this clade will be provided



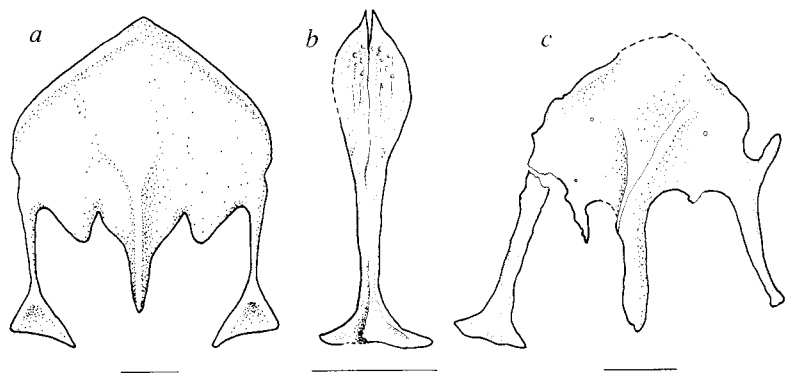
elsewhere. *Eoalulavis* is diagnosed by the presence of cervical and dorsal laminar, keel-like vertical centra; undulated ventral surface of furcula; distal end of humerus with thick, caudally projected ventral margin; several small tubercles on the distal, caudal surface of the minor metacarpal; depressed, lanceolate sternum, with foot-like caudal expansion, and a faint carina; and a deep, rostral cleft in the sternum.

modern birds for low-speed flight and manoeuvrability. To decrease their speed while maintaining lift on the wings, birds must increase the angle of attack of their wings (Fig. 4b). This leads to an increase in drag. There is a limit to the angle of attack, for when the angle is large the wing stalls: turbulent eddies develop over the dorsal surface of the wings, and lift drops dramatically so that the bird can no longer fly. The alula, which acts as a slot on the medial leading edge of the wing, serves to delay stall and to allow the wing to continue to generate lift even at very high angles of attack. The alula lifts automatically when pressure above the wing drops, and in most birds is used only in very slow flight, take off and landing¹¹⁻¹³.

Eoalulavis is the most primitive bird found with an alula. The fact that this structure is absent in the well-preserved, feathered wings of the Berlin and Eichstätt specimens of *Archaeopteryx* suggests that this may reflect the Urvogel's actual condition. There has been much debate about the aerodynamic capability of *Archaeopteryx*, but no consensus has been achieved other than the acceptance that it was able to perform some sort of flight^{6,14-19}.

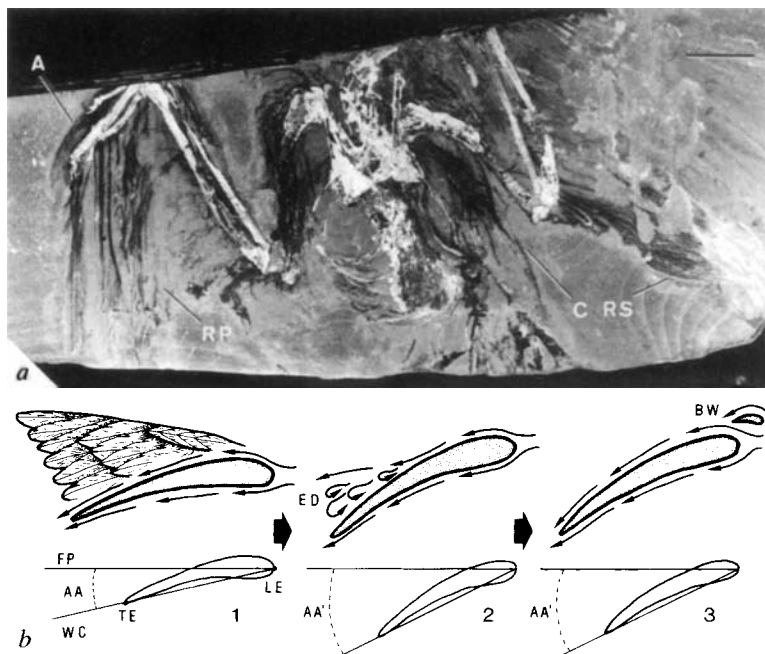
If the alula was truly absent, *Archaeopteryx* may have had aerodynamic constraints during low speed flights²⁰, unless it eventually possessed an alternative means of performing this kind of flight. Furthermore, *Archaeopteryx* lacks most modern skeletal structures usually correlated with an enhanced flying ability. It lacks a strut-like coracoid, a rigid carpometacarpus and a pygostyle, and it retains proportions between wing elements typical of its flightless, non-avian theropod relatives^{21,22}. Indeed, the basic design of the modern flight apparatus is first known for the basalmost ornithothoracine birds such as the Early Cretaceous *Iberomesornis*^{1,2}. Structural disparity between *Archaeopteryx* and basal ornithothoracines indicates important differences in their ability to fly. In *Eoalulavis*, an advanced skeletal design of the flight apparatus is consorted with a novel element in feather function. This suggests that basal ornithothoracines had substantially improved their flight capabilities. *Eoalulavis* demonstrates that as early as 115 million years ago, birds had developed a sophisticated structural complex allowing them to perform low-speeds flights and accurate manoeuvrability control. □

FIG. 3 Comparisons between the sternum of *Eoalulavis* and that of other Enantiornithes. Scale bars represent 5 mm. a, Sternum of *Cathayornis yandica* (modified from ref. 25) in ventral view. b, Sternum of *Eoalulavis hoyasi* in ventral view. c, Sternum of *Concornis lacustris* (modified from ref. 4) in ventral view. The sternum of *Eoalulavis* stands out among the sterna of other enantiornithines because it is remarkably narrow and it possesses only a faint ventral keel. This condition, which may suggest a reduced volume of flight musculature, contrasts with the many other features indicating aerodynamic skills. Nevertheless, the tall coracoids could have been connected to the robust furcula by a coraco-clavicular membrane providing an extensive area for the origin of the flight musculature, a condition previously suggested for *Archaeopteryx*²⁶. Moreover, the absence of sternal facets for the articulation with the coracoids suggests that the preserved bone may be the remnant of a larger cartilaginous structure which may have provided additional area for the origin of the flight muscles (there is increasing evidence for the existence of a widely unossified large sternum in early birds, including *Archaeopteryx*). Even if so, we believe that the caudal expansion and the rostral notch of the sternum



represent its actual shape. Hence these features are considered here as autapomorphies of *Eoalulavis*. The extremely long hypocleidium presumably fits into the rostral sternal notch, providing further strength to the thoracic girdle.

FIG. 4a, Ultraviolet induced fluorescence photograph (before preparation). Most of the isolated feathers from Las Hoyas are preserved as carbonized traces, the most common type of feather preservation in the fossil record²⁷. Nevertheless, the feathers of *Eoalulavis* are usually highlighted (like some bones) by limonite and, occasionally, by pyroluxite. *Eoalulavis* lacks a true carpometacarpus, as the distal ends of the major and minor metacarpals are not fused to each other. It is not possible to verify the condition of the wrist. Despite the conclusions of ref. 19, the absence of a true carpometacarpus does not seem to preclude active flight, considering the presence in *Eoalulavis* of an alula and other advanced skeletal features. Scale bar represents 1 cm. Abbreviations: A, alula; C, body feathers; RP, primary remiges; RS: secondary remiges. b, Schematic drawing of a cross section of a wing with (1) a small angle of attack, (2) a large angle of attack without an alula, and (3) a large angle of attack with an extended alula. Abbreviations: AA, angle of attack; AA', increased AA; BW, alula or bastard wing; ED, eddying; FP, flight path; LE, leading edge; TE, trailing edge; WC, wing chord. Modified from ref. 28.



Received 22 March; accepted 6 June 1996.

1. Sanz, J. L. & Bonaparte, J. F. in *Papers in Avian Paleontology Honoring Pierce Brodkorb* (ed. Campbell, K. E. Jr) 39–49 (Nat. Hist. Mus. Los Angeles, Sci. Ser. 36, Los Angeles, 1992).
2. Sanz, J. L., Bonaparte, J. F. & Lacasa, A. *Nature* **331**, 433–435 (1988).
3. Sanz, J. L. & Buscalioni, A. D. *Palaeontology* **35**, 829–845 (1992).
4. Sanz, J. L., Chiappe, L. M. & Buscalioni, A. D. *Am. Mus. Novit.* **3133**, 23 (1995).
5. Fregenal-Martínez, M. A. & Meléndez, N. in *Las Hoyas: A Lacustrine Konservat-Lagerstätte, Cuenca, Spain. Field Trip Guide Book* (ed. Meléndez, N.) 1–10 (Universidad Complutense de Madrid, Spain, 1995).
6. Chiappe, L. M. *Nature* **378**, 349–355 (1995).
7. Chiappe, L. M. & Calvo, J. O. *J. Vert. Paleont.* **14**, 230–246 (1994).
8. Pérez-Moreno, B. P., Sanz, J. L., Buscalioni, A. D., Moratalla, J. J., Ortega, F. & Rasskin-Gutman, D. *Nature* **370**, 363–367 (1994).
9. McGowan, G. & Evans, S. E. *Nature* **373**, 143–145 (1995).
10. Martin, L. D. & Tate, J. Jr *Smithson. Contr. Paleobiol.* **27**, 35–66 (1976).
11. Nachtigall, W. & Kempf, B. Z. *vergl. Physiol.* **71**, 326–341 (1971).
12. Graham, R. R. *J. R. aero. Soc.* **36**, 598–600 (1932).
13. Brown, R. H. *J. Biol. Rev.* **38**, 460–489 (1963).
14. Hecht, M. K., Ostrom, J. H., Viol, G. & Wellenhofer, P. (eds) *The Beginnings of Birds* (Jura Museum, Eichstätt, 1985).
15. Feduccia, A. *Science* **259**, 790–793 (1993).
16. Speakman, J. R. *Evolution* **47**, 336–340 (1993).
17. Speakman, J. R. & Thomson, S. C. *Nature* **370**, 514 (1994).
18. Norberg, R. Å., Speakman, J. R. & Thomson, S. C. *Nature* **374**, 221–222 (1995).

19. Vázquez, R. J. *J. Morphol.* **211**, 259–268 (1992).
20. Rayner, J. M. V. in *Biomechanics in Evolution* (eds Rayner, J. M. V. & Wootton, R. J.) 183–212 (Cambridge Univ. Press, 1991).
21. Ostrom, J. H. *Biol. J. Linn. Soc. Lond.* **8**, 91–182 (1976).
22. Chiappe, L. M. *Müchuer Geowissenschaftliche Abhandlungen (A)* **30**, 203–244 (Verlag Dr Friedrich Pfeil, 1966).
23. Maisey, J. G., Rutzky, I., Blum, S. & Elvers, W. in *Santana Fossils. An Illustrated Guide* (ed. Maisey, J. G.) 98–105 (T.F.H. Publications, New York, 1991).
24. Farns, J. Hennig 86 references. *Documentation for version 1.5*. (Privately published, 1988).
25. Zhou, Z. *Cour. Forschungsinst. Senckenberg* **181**, 9–22 (1995).
26. Olson, S. L. & Feduccia, A. *Nature* **278**, 247–248 (1979).
27. Davis, P. G. & Briggs, D. E. G. *Geology* **23**, 783–786 (1995).
28. Spearman, R. I. C. & Hardy, J. A. in *Form and Function in Birds Vol. 3* (eds King, A. S. & McLelland, J.) 1–56 (Academic, London, 1985).

ACKNOWLEDGEMENTS. We thank K. Padian, J. M. V. Rayner and L. D. Martin for their valuable suggestions and comments, and P. Dandonoli and D. Rasskin-Gutman for revising the manuscript. E. García-Raso and E. E. Pinardo-Moya analysed the digestive remains and photographs were taken by G. F. Kurtz. This work was supported by Junta de Comunidades de Castilla-La Mancha, DGICYT (Promoción General del Conocimiento), the EU (Human Capital and Mobility Program) and grants from the NSF and Guggenheim Foundation to L.M.C.

CORRESPONDENCE and requests for materials should be addressed to J.L.S. (e-mail: nino@ccuam3.sdi.uam.es).

Humic substances as electron acceptors for microbial respiration

Derek R. Lovley*, John D. Coates†, Elizabeth L. Blunt-Harris*, Elizabeth J. P. Phillips† & Joan C. Woodward*†

* Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003, USA

† Water Resources Division, US Geological Survey, Reston, Virginia 22092, USA

HUMIC substances are heterogeneous high-molecular-weight organic materials which are ubiquitous in terrestrial and aquatic environments. They are resistant to microbial degradation¹ and thus are not generally considered to be dynamically involved in microbial metabolism, especially in anoxic habitats. However, we show here that some microorganisms found in soils and sedi-

ments are able to use humic substances as an electron acceptor for the anaerobic oxidation of organic compounds and hydrogen. This electron transport yields energy to support growth. Microbial humic reduction also enhances the capacity for microorganisms to reduce other, less accessible electron acceptors, such as insoluble Fe(III) oxides, because humic substances can shuttle electrons between the humic-reducing microorganisms and the Fe(III) oxide. The finding that microorganisms can donate electrons to humic acids has important implications for the mechanisms by which microorganisms oxidize both natural and contaminant organics in anaerobic soils and sediments, and suggests a biological source of electrons for humics-mediated reduction of contaminant metals and organics.

In a study on the effect of various Fe(III) chelators on anaerobic benzene degradation in petroleum-contaminated aquifers, it was found that humic acids stimulated benzene degradation better than any of the synthetic chelators evaluated². Synthetic chelators stimulate benzene degradation by solubilizing Fe(III) oxides and thus making Fe(III) more available to benzene-oxidizing Fe(III)-reducing bacteria^{3,4}. The superiority of the humic acids over synthetic chelators such as nitrilotriacetic acid (NTA) was surprising because although humic acids can chelate Fe(III)⁵, they do not

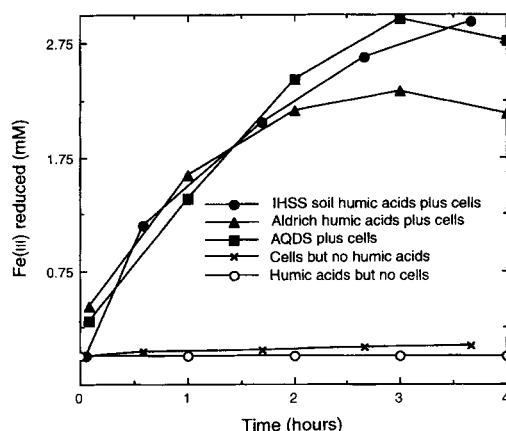


FIG. 1 Fe(III) reduction to Fe(II) by cell suspensions of *G. metallireducens*. Addition of soil humic acid from the IHSS or commercially available Aldrich humic acids greatly stimulated Fe(III) reduction, as did the humic analogue 2,6-anthraquinone disulphonate (AQDS).

METHODS. Washed cells of *G. metallireducens* (0.4, 1.3 or 1.3 mg protein per ml for IHSS soil humic acids, Aldrich humic acids and AQDS respectively) were suspended in 10 ml sodium bicarbonate buffer (30 mM; pH 6.8) under N_2-CO_2 (80:20) containing acetate (10 mM) as the electron donor and synthetic, poorly crystalline Fe(III) oxide (~ 6 mM)²² as the electron acceptor. The incubation temperature was 30 °C. Subsamples were analysed for Fe(II) with ferrozine in HEPES buffer²². The concentration of dissolved humic acids was 2 mg ml⁻¹ and of AQDS was 100 μ M.

have the chelation capacity of synthetic chelators.

To determine whether humic acids stimulated microbial Fe(III) reduction as previously shown for synthetic chelators^{3,4}, cell suspensions of the dissimilatory Fe(III)-reducing bacterium *Geobacter metallireducens* were added to bicarbonate buffer containing acetate as the electron donor and poorly crystalline Fe(III) oxide as the electron acceptor. Cell suspensions of *G. metallireducens* only slowly reduced Fe(III) oxide⁴, but when humic acids were also added to the buffer, Fe(III) reduction was greatly stimulated (Fig. 1). The same degree of stimulation was observed with highly purified 'soil humic acids' supplied by the International Humic Substances Society (IHSS) and with the commercially

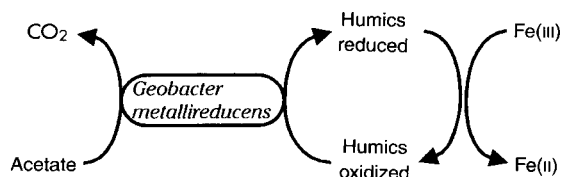


FIG. 2 Model for mechanism by which humic acids stimulate Fe(III) reduction by *G. metallireducens*.

available Aldrich humic acids that were previously found to stimulate benzene metabolism². Humic acids did not reduce appreciable amounts of Fe(III) when *G. metallireducens* was omitted (Fig. 1).

Stimulation of microbial Fe(III) reduction by NTA is associated with visible dissolution of Fe(III) oxide and yields millimolar concentrations of dissolved Fe(III)⁴. In contrast, the humic acids did not visibly dissolve Fe(III) oxide and the concentration of dissolved Fe(III) in the presence of humic acids but without added cells was less than 200 μ M. This suggested that the mechanism by which humic acids stimulated Fe(III) reduction was different from that for synthetic Fe(III) chelators.

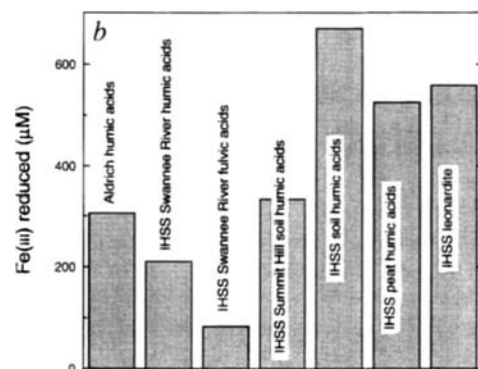
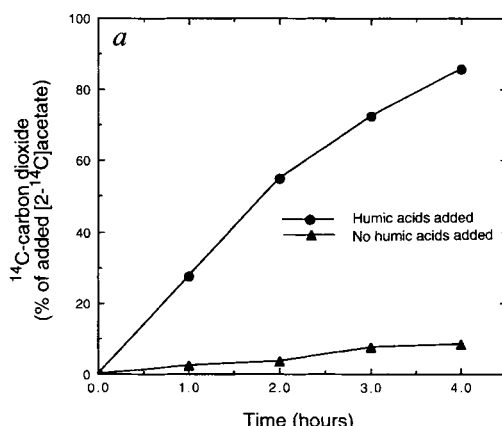
An additional property of humic acids is their ability to undergo reduction-oxidation. For example, humic substances and quinone-containing humic analogues can transfer electrons from reduced inorganics (sulphide) and organics (ascorbic acid) to contaminants such as mercury^{6,7}, nitroaromatics^{8,9} and chlorinated solvents¹⁰. Although direct reduction of humic acids by microorganisms has not been demonstrated⁷, it is well known that reduced humic acids can donate electrons to Fe(III)¹¹⁻¹³.

We speculated that if *G. metallireducens* could transfer electrons to humic substances, then humic substances might stimulate Fe(III) reduction in a two-stage process in which (1) *G. metallireducens* oxidizes acetate, with humic acids acting as the electron acceptor, and (2) reduced humic acids donate electrons to Fe(III) (Fig. 2). Such electron shuttling would alleviate the need for Fe(III) reducers to contact Fe(III) oxides directly in order to reduce them and so would accelerate Fe(III) reduction.

To evaluate step (1) in the model, cells of *G. metallireducens* were suspended in buffer containing acetate as the sole electron donor and humic acids as a potential electron acceptor (Fig. 3a). Acetate was readily oxidized to carbon dioxide in the presence of

FIG. 3 Data supporting the two-step model for humic stimulation of Fe(III) reduction. a, Humic acid-dependent oxidation of acetate by *G. metallireducens*, and b, the ability of humic substances reduced by *G. metallireducens* to reduce Fe(III).

METHODS. a, Washed cell suspensions of *G. metallireducens* (1 mg protein per ml) were added to 10 ml bicarbonate buffer (as for Fig. 1) which contained acetate (0.22 mM) containing [2-¹⁴C]acetate (1 μ Ci) as electron donor. Production of ¹⁴CO₂ was determined as described³. IHSS soil humic acids were added at 2 mg ml⁻¹. b, Washed cell suspensions of *G. metallireducens* containing acetate as electron donor and the specified humic substance (2 mg ml⁻¹) as potential electron acceptor were incubated for 2 h. Cells were removed by filtration (0.2- μ m-pore-diameter filter) in an anaerobic glove bag and an anaerobic stock of Fe(III) citrate was added to



10 mM final concentration. After 15 min, subsamples were taken for Fe(II) determination. Poorly crystalline Fe(III) oxide was also reduced by reduced humic acids, but Fe(III) citrate was used here for easier quantification. With the exception of the Aldrich humic acids, all of the humics substances evaluated were highly purified samples obtained from the IHSS.

humics, but there was little oxidation in the absence of humic acids. There was no acetate oxidation in the presence of humic acids when *G. metallireducens* was omitted, showing that humic acids can serve as an electron acceptor for acetate oxidation by *G. metallireducens*.

To determine whether the electrons transferred from acetate to humic acids by *G. metallireducens* could then be transferred to Fe(III) (step (2) of the model), cell suspensions were incubated with humic substances and the cells removed by filtration. When Fe(III) was added to the filtrates, the Fe(III) was reduced (Fig. 3b). This was true for a wide variety of highly purified humic substances obtained from IHSS as well as for the commercially available Aldrich humic acids. As in Fig. 1, humic substances not incubated with *G. metallireducens* did not reduce Fe(III). Furthermore, in accordance with a previous demonstration that *G. metallireducens* does not release extracellular components capable of reducing Fe(III)¹⁴, filtrates of cell suspensions that did not contain humic acids did not reduce Fe(III). Another Fe(III)-reducing micro-organism, *Shewanella alga*, which can reduce Fe(III) with H₂ or by incompletely oxidizing lactate to acetate^{15,16}, was found to transfer electrons to soil humic acids in a comparable way, with lactate or H₂ serving as electron donor (data not shown).

To quantify the electron transfer from acetate to humics and then from humics to Fe(III), the amount of acetate oxidized (Fig. 3a) and the amount of Fe(III) reduced by humics to Fe(II) (Fig. 3b) were determined simultaneously on cell suspensions incubated with soil humic acids for 1.5 h. The ratio of the number of moles of Fe(II) produced by the reduced humics to the number of moles of acetate oxidized in the presence of humics was 8.3 ± 0.7 (mean $\pm$ s.d.; $n = 3$). This compares favourably with the reduction of Fe(III) to Fe(II) by humics, which requires one electron, and the acceptance by humics of eight electrons per mol of acetate oxidized by *G. metallireducens*. This result indicates that electrons transferred to humic acids from acetate by *G. metallireducens* can be quantitatively transferred to Fe(III). In comparable experiments with *S. alga*, the amount of Fe(II) produced was $100 \pm 10\%$ ($n = 3$) of that expected for complete transfer of electrons from lactate to humics and then from humics to Fe(III).

The ability of humic acids to participate in abiological electron transfer has suggested that quinone moieties may be the electron-accepting groups, with the resultant hydroquinones donating electrons to the ultimate electron acceptor^{9,10}. As the complexity of humic acid structure has precluded direct proof of this, the potential involvement of the quinone/hydroquinone redox couple in such electron transfer has been investigated with model compounds such as 2,6-anthraquinone disulphonate (AQDS)^{10,17}.

Concentrations of AQDS as low as 100 μ M greatly stimulated Fe(III) reduction by cell suspensions of *G. metallireducens* (Fig. 1). There was no detectable solubilization of Fe(III) oxide by the AQDS. Once Fe(III) reduction was complete, the buffer turned orange owing to the accumulation of reduced AQDS (namely, 2,6-anthrahydroquinone disulphonate (AHDS). If more Fe(III) was added, then the orange colour immediately disappeared. When Fe(III) was added to known concentrations of AHDS in anaerobic buffer, the orange colour again disappeared immediately and there was an accumulation of Fe(II) that was consistent with the oxidation of AHDS back to AQDS, with reduction of 2 mol of Fe(III) per mol of AHDS oxidized. These results show that *G. metallireducens* can reduce AQDS to AHDS, which in turn can instantaneously reduce Fe(III) and regenerate AQDS.

Both *G. metallireducens* and *S. alga* could grow in medium containing AQDS as the sole electron acceptor (Fig. 4a, b). Growth coincided with AQDS reduction. There was no growth or AQDS reduction if the electron donor was omitted (Fig. 4a, b), or if the electron donors were provided but the AQDS omitted (data not shown). The stoichiometry of acetate uptake and AQDS reduction during growth on *G. metallireducens* (Fig. 4a) was consistent with oxidation of acetate and reduction of AQDS according to: $\text{acetate}^- + 4\text{H}_2\text{O} + 4\text{AQDS} \rightarrow 4\text{AHDS} + 2\text{HCO}_3^- + \text{H}^+$; in cultures of *S. alga*, lactate consumption and AQDS reduction (Fig. 4b) was in

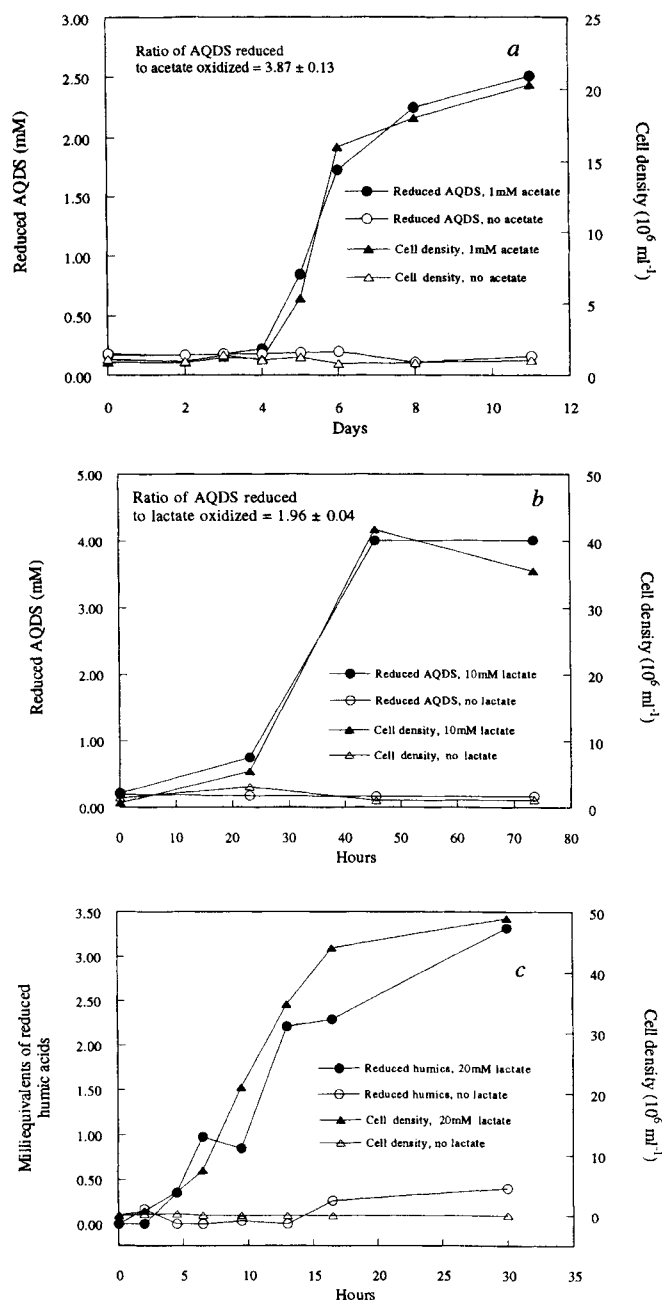


FIG. 4 Growth of *G. metallireducens* and *S. alga* with 2,6-anthraquinone disulphonate (AQDS) (a, b) or humic acids (c) as electron acceptor.

METHODS. Cells were inoculated into anaerobic freshwater medium¹⁴ containing acetate (*G. metallireducens*) or lactate (*S. alga*) as electron donor and AQDS or humic acids as potential electron acceptor. Reduction of AQDS was quantified by the absorbance of 2,6-anthrahydroquinone disulphonate (AHDS) produced at 450 nm. In separate incubations, the stoichiometry of acetate or lactate consumption and AHDS formation were determined by measuring loss of electron donors using high-performance liquid chromatography. Millequivalents of reduced humic acids produced were estimated from the amount of Fe(III) reduced when a filtrate of a subsample was exposed to Fe(III), as for Fig. 3. Cell growth on AQDS was monitored by direct cell counting¹⁴. Monitoring cell growth by direct cell counting or by protein determination was not feasible when the organisms were grown on humic acids as electron acceptor because of interference by the humic acids. Therefore, cell numbers of *S. alga* were determined from plate counts on aerobic heterotrophic medium. This method could not be used with the strict anaerobe *G. metallireducens*, so growth of this organism on humic acids could not be quantified.

agreement with the reaction: $\text{lactate}^- + 2\text{H}_2\text{O} + 2\text{AQDS} \rightarrow 2\text{AHDS} + \text{acetate}^- + \text{HCO}_3^- + \text{H}^+$.

Technical difficulties prevented cell growth of *G. metallireducens* from being monitored with humic acids as the electron acceptor (Fig. 4); however, *S. alga* grew in a medium in which humic acids were the sole electron acceptor (Fig. 4c). Growth was associated with an accumulation of reducing potential in the humic acids that could be transferred to Fe(III) when Fe(III) was added to cell-free filtrates of the culture. Growth was not due to degradation by *S. alga* of humic acids because there was no growth in the presence of humic acids if lactate, the electron donor, was omitted (Fig. 4c). These results show that respiration with humic acids as the terminal electron acceptor can yield energy to support cell growth.

Besides revealing a new form of microbial respiration, these findings may have important implications for the biogeochemistry of soils and aquatic sediments. For example, the reduction of insoluble Fe(III) and Mn(IV) oxides is one of the most geochemically significant processes that takes place in sedimentary environments^{18,19}. Previous investigations into the mechanisms for the reduction of Fe(III) and Mn(IV) have emphasized either the abiological reduction of these metals by organics such as humic materials and related aromatic compounds²⁰, or the direct biological reduction of the metals by specialized metal-reducing microorganisms²¹. Our results suggest that, at least in some instances, the reduction of Fe(III) (and other metals such as Mn(IV) that can also accept electrons from humic substances) may actually be a combination of both processes, with microorganisms first donating electrons to humic substances and the humic substances then reducing the metals. Such electron shuttling may greatly facilitate the ability of Fe(III)-reducing bacteria to pass electrons to insoluble Fe(III) oxides and is the likely explanation for the earlier observation² that humic acids greatly accelerate the rate of benzene degradation in aquifer sediments in which Fe(III) is the terminal electron acceptor. Even when Fe(III)

is not available to recycle humic substances back to their oxidized form, humic substances may still be important electron acceptors for organic matter oxidation, given the abundance of humic substances in many soils and sediments. The known ability of reduced humic acids to donate electrons to a variety of metals and organics^{6,9,10,12} suggests that microbial reduction of humic acids may have an impact on the fate of other environmental contaminants as well. □

Received 7 May; accepted 14 June 1996.

- McKnight, D. M. et al. in *Organic Acids in Aquatic Ecosystems* (eds Perdue, E. M. & Gjessing, E. T.) 223–243 (Wiley, New York, 1990).
- Lovley, D. R., Woodward, J. C. & Chapelle, F. H. *Appl. Environ. Microbiol.* **62**, 288–291 (1996).
- Lovley, D. R., Woodward, J. C. & Chapelle, F. H. *Nature* **370**, 128–131 (1994).
- Lovley, D. R. & Woodward, J. C. *Chem. Geol.* (in the press).
- Jackson, K. S., Jonasson, I. R. & Skippen, G. B. *Earth Sci. Rev.* **14**, 97–146 (1978).
- Alberts, J. J., Schindler, J. E., Miller, R. W. & Nutter, D. E. *Science* **184**, 895–897 (1974).
- Schindler, J. E., Williams, D. J. & Zimmerman, A. P. in *Environmental Biogeochemistry* Vol. 1 (eds Nriagu, J. O.) 109–115 (Ann Arbor Science, Ann Arbor, Michigan, 1976).
- Schwarzenbach, R. P., Stierli, R., Lanz, K. & Zeyer, J. *Environ. Sci. Technol.* **24**, 1566–1574 (1990).
- Dunnivant, F. M., Schwarzenbach, R. P. & Macalady, D. L. *Environ. Sci. Technol.* **26**, 2133–2142 (1992).
- Curtis, C. P. & Reinhard, M. *Environ. Sci. Technol.* **28**, 2393–2401 (1994).
- Szilagyi, M. *Soil Sci.* **111**, 233–235 (1971).
- Skogerboe, R. K. & Wilson, S. A. *Analyt. Chem.* **53**, 228–232 (1981).
- Kahn, T. R., Langford, C. H. & Skippen, G. B. *Org. Geochem.* **7**, 261–266 (1984).
- Lovley, D. R. & Phillips, E. J. P. *Appl. Environ. Microbiol.* **54**, 1472–1480 (1988).
- Caccavo, F. Jr, Blakemore, R. P. & Lovley, D. R. *Appl. Environ. Microbiol.* **58**, 3211–3216 (1992).
- Rossello-Mora, R. A. et al. *Syst. appl. Microbiol.* **17**, 569–573 (1994).
- Tratnyek, P. G. & Macalady, D. L. *J. Agric. Food Chem.* **37**, 248–254 (1989).
- Ponnampuram, F. N. *Adv. Agron.* **24**, 29–96 (1972).
- Lovley, D. R. *Adv. Agron.* **54**, 175–231 (1995).
- LaKind, J. S. & Stone, A. T. *Geochim. cosmochim. Acta* **53**, 961–971 (1989).
- Lovley, D. R., Phillips, E. J. P. & Lonergan, D. J. *Environ. Sci. Technol.* **25**, 1062–1067 (1991).
- Lovley, D. R. & Phillips, E. J. P. *Appl. Environ. Microbiol.* **51**, 683–689 (1986).

ACKNOWLEDGEMENTS. We thank D. McKnight and P. Tratnyek for helpful discussions. This research was supported by the Office of Naval Research and by the American Petroleum Institute.

CORRESPONDENCE and requests for materials should be addressed to D.R.L. (e-mail: dlovley@microbio.umass.edu).

Increased bone formation in osteocalcin-deficient mice

Patricia Ducy, Christelle Desbois, Brendan Boyce*, Gerald Pinero†, Beryl Story*, Colin Dunstan‡, Erica Smith§, Jeffrey Bonadio||, Steven Goldstein§, Caren Gundberg¶, Allan Bradley# & Gerard Karsenty

Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Box 45, Houston, Texas 77030, USA
Departments of * Pathology, and ‡ Medicine, The University of Texas Health Science Center, San Antonio, Texas 78284, USA

† Department of Basic Science, Dental Branch University of Texas, Houston, Texas 77030, USA

Departments of § Orthopaedics, and || Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA

¶ Department of Orthopaedics, Yale School of Medicine, New Haven, Connecticut 06510, USA

Howard Hughes Medical Institute and Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA

VERTEBRATES constantly remodel bone. The resorption of pre-existing bone by osteoclasts and the formation of new bone by osteoblasts is strictly coordinated to maintain bone mass within defined limits. A few molecular determinants of bone remodelling that affect osteoclast activity^{1–3} have been characterized, but the molecular determinants of osteoblast activity are unknown. To investigate the role of osteocalcin, the most abundant osteoblast-specific non-collagenous protein⁴, we have gen-

erated osteocalcin-deficient mice. These mice develop a phenotype marked by higher bone mass and bones of improved functional quality. Histomorphometric studies done before and after ovariectomy showed that the absence of osteocalcin leads to an increase in bone formation without impairing bone resorption. To our knowledge, this study provides the first evidence that osteocalcin is a determinant of bone formation.

Osteocalcin, the most abundant non-collagenous protein (NCP) of the bone extracellular matrix (ECM), is synthesized only by osteoblasts^{5,6}. The mouse osteocalcin cluster contains three genes, two of which (*OG1* and *OG2*) encode osteocalcin. Their coding sequences are 96% identical, and they are expressed only by osteoblasts, whereas the third gene, osteocalcin-related gene (*ORG*), is expressed in kidney but not in bone⁷. To investigate the function of osteocalcin during bone remodelling, osteocalcin-deficient mice (*osc^{ml}/osc^{ml}*) were generated by simultaneously deleting both *OG1* and *OG2* using embryonic stem (ES) cell technology (Fig. 1). Heterozygous mice were phenotypically normal. Crosses between these heterozygotes produced homozygous mutant *osc^{ml}/osc^{ml}* mice at predicted mendelian frequencies. Because both *OG1* and *OG2* were deleted, osteocalcin was not expressed in these mice (Fig. 1d–f). There was no ectopic expression of *ORG* in bone (Fig. 1d), indicating that *osc^{ml}* is a null allele for osteocalcin. These mice were normal at birth, viable and fertile, and had no skeletal-patterning defects and no ectopic bone formation. The expression of other NCPs such as osteopontin, matrix gla protein, and bone sialo protein was not significantly affected by the absence of osteocalcin (Fig. 1g and data not shown). The ultrastructure of the bone ECM of 3- and 6-month-old wild-type and mutant animals examined by electron microscopy was identical (data not shown).

Over time the mutant mice developed abnormalities of bone remodelling which became noticeable in 6-month-old animals. Radiographic analysis of the long bones of 6-month-old wild-type and mutant animals revealed that the mutant long bones had increased cortical thickness and density compared to wild-type littermates, consistent with an increased amount of mineralized bone matrix (Fig. 2a). In the bones of 9-month-old animals, the same features were present and were accompanied by an increase in the width of the diaphysis (Fig. 2b).

Histological analysis of 6- and 9-month-old animals showed the presence of more cancellous bone in mutant animals than in wild-type littermates (Fig. 2c, d and data not shown). The width of cortical bone in 6-month-old mutant mice was increased compared with that of their wild-type littermates, reaching 150% of the wild-type width by 9 months of age (*osc^{ml}/osc^{ml}* mice,

257 ± 28 µm; *Osc⁺/Osc⁺*, 169 ± 20 µm, *n* = 3; Fig. 2g-j). The functional consequences of this increase in bone mass were analysed biomechanically. In 6-month-old mutant mice, a significant increase in failure load, a biomechanical indicator of bone strength⁸, was observed compared with that of wild-type littermates (*osc^{ml}/osc^{ml}* mice, 33.79 ± 5.41 N; *Osc⁺/Osc⁺*, 27.08 ± 3.25 N; *P* < 0.05 ANOVA, *n* = 6). This result indicates that the increase in bone tissue had a beneficial effect on the mechanical properties of the bones.

To determine whether this increase in bone mass was due to an increase in bone formation, histomorphometry analysis was performed by double-labelling with tetracycline, a marker of the amount of newly formed bone⁹. At both 4.5 and 6 months of age there was a 50% increase (*P* < 0.05, *n* = 4) in the percentage of labelled surface in mutant animals compared to wild-type

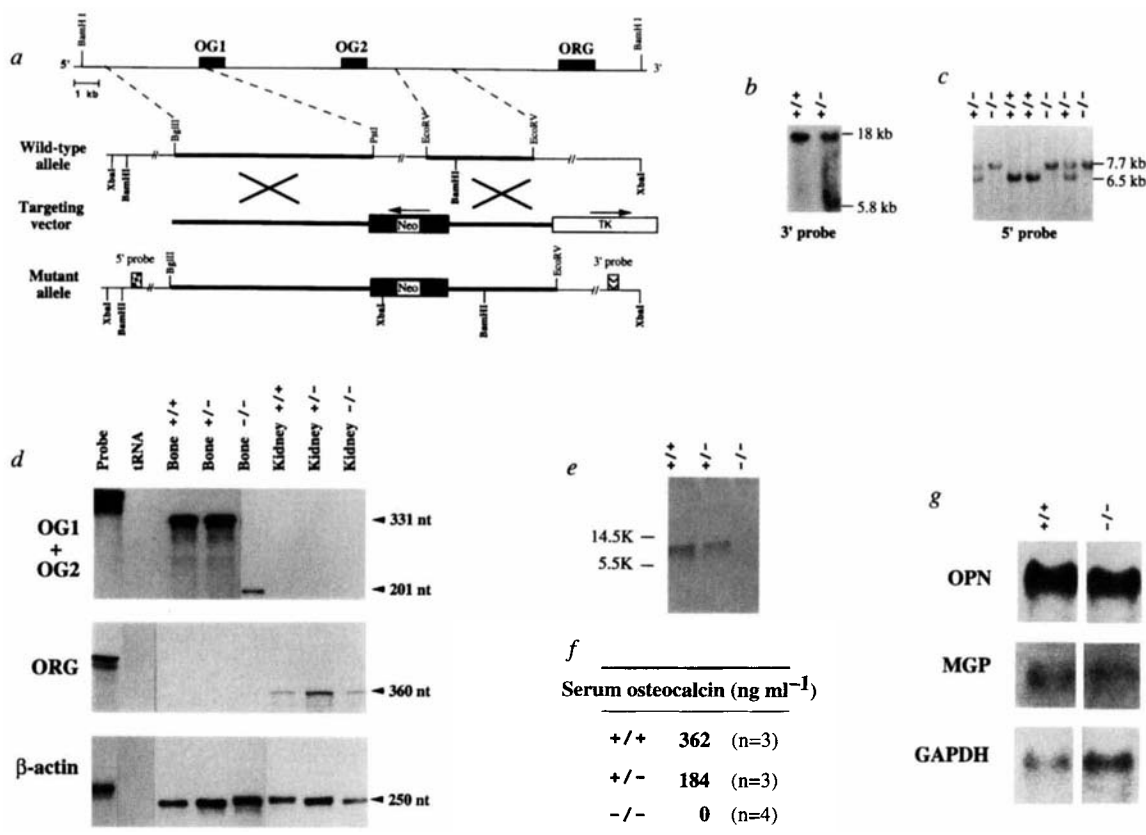


FIG. 1 Gene targeting at the osteocalcin locus. **a**, Representation of the expected gene replacement at the mouse *Osteocalcin* locus⁷ (*Osc*). Exon 4 of osteocalcin gene 1 (OG1), which codes for the mature protein, and the entire osteocalcin gene 2 (OG2) sequence were deleted, while osteocalcin-related gene (ORG) was retained. Correct targeting resulted in the replacement of the entire mature osteocalcin protein-coding sequences by the pGKNeo cassette¹. The MC1-tk (thymidine kinase) expression cassette¹⁶ was used for negative selection. The 5' and 3' external probes used for Southern-blot analysis are shown as patterned boxes. **b**, Southern blot analysis of targeted ES cell DNA. The presence of a 5.8-kb *Xba*I fragment indicates proper targeting of the *Osc* locus at the 3' end. **c**, Southern blot analysis of DNA from the offspring of heterozygous matings. Genomic DNA (5 µg) isolated from the tails of one litter was digested with *Bam*HI and analysed with the 5' probe. The presence of a single 7.7-kb fragment indicates a homozygous mutant (-/-) genotype. **d**, RNase protection analysis of RNA from the offspring of heterozygous matings. 15 µg of total RNA collected from bone or kidney was hybridized with a labelled riboprobe corresponding to mouse osteocalcin cDNA (OG1 + OG2) or to the first exon of ORG (ORG). Arrowheads denote the positions of the expected protected products. The 201-nucleotide (nt) band observed with the OG1/OG2 probe in homozygote mutant animals corresponds to the expected protected product of the first three exons of OG1. tRNA was used as a

negative control. 5 µg of RNA from bone and kidney was hybridized with a control β-actin riboprobe. **e**, Western blot analysis of protein from the bone ECM of wild-type (+/+), heterozygote (+/-), and homozygote mutant (-/-) mice. **f**, Radioimmunoassay of serum osteocalcin in wild-type (+/+), heterozygote (+/-), and homozygous mutant (-/-) animals. Numbers in parentheses indicate numbers of animals tested. **g**, Northern blot analysis of RNA from 6-week-old wild-type (+/+) and homozygote mutant (-/-) mice. 15 µg of total RNA from bone was hybridized with a labelled probe corresponding either to the mouse matrix gla protein cDNA¹⁷ (MGP) or to the rat osteopontin cDNA¹⁸ (OPN). A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used to normalize the quantity of RNA in each lane.

METHODS. Culture and transfection of ES cells have been described¹⁹. Briefly, AB2.1 ES cells were transfected with 25 µg linearized targeting vector per 10⁷ cells with a Biorad Gene Pulser and grown under double selection^{1,16}. Targeted ES cell clones were identified by Southern blot hybridization and were injected into C57BL/6 blastocysts²⁰. Male chimaeras were mated to C57BL/6 females and their heterozygous offspring were intercrossed to generate *osc^{ml}/osc^{ml}* mice. RNase protection assays were done with an RPA II kit (Ambion), following the manufacturer's instructions. Radioimmunoassay and western blot analysis were done with an anti-mouse osteocalcin antibody²¹.

FIG. 2 Radiological and histological analysis of the bones of *osc^{m1}/osc^{m1}* mice. **a, b**, X-ray of femora of wild-type (+/+) and mutant (-/-) littermates of 6 (**a**) and 9 months old (**b**). Note the denser aspect and increase in cortical thickness of the long bones of mutant compared with wild-type animals. The head of the femur of the 9-month-old mutant mouse was sectioned for histological analysis in a separate experiment. **c, d**, Histological analysis of cancellous bone in 9-month-old wild-type (**c**) and *osc^{m1}/osc^{m1}* mice (**d**). Longitudinal sections were made through the femora. Note the abundance of calcified cancellous bone surrounding the growth-plate cartilage. **e, j**, Histological analysis of cortical bones of wild-type (**e, g, i**) and *osc^{m1}/osc^{m1}* mice (**f, h, j**) of 3 (**e, f**), 6 (**g, h**), or 9 months old (**i, j**). Longitudinal sections are through the femora. Note the progressive increase in cortical thickness in the bones of mutant mice.

METHODS. Mice were killed by cervical dislocation, and tissues were fixed in fresh 4% paraformaldehyde for 10 to 24 h. Samples were demineralized for

2 days in 10% formic acid, dehydrated, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin/eosin.

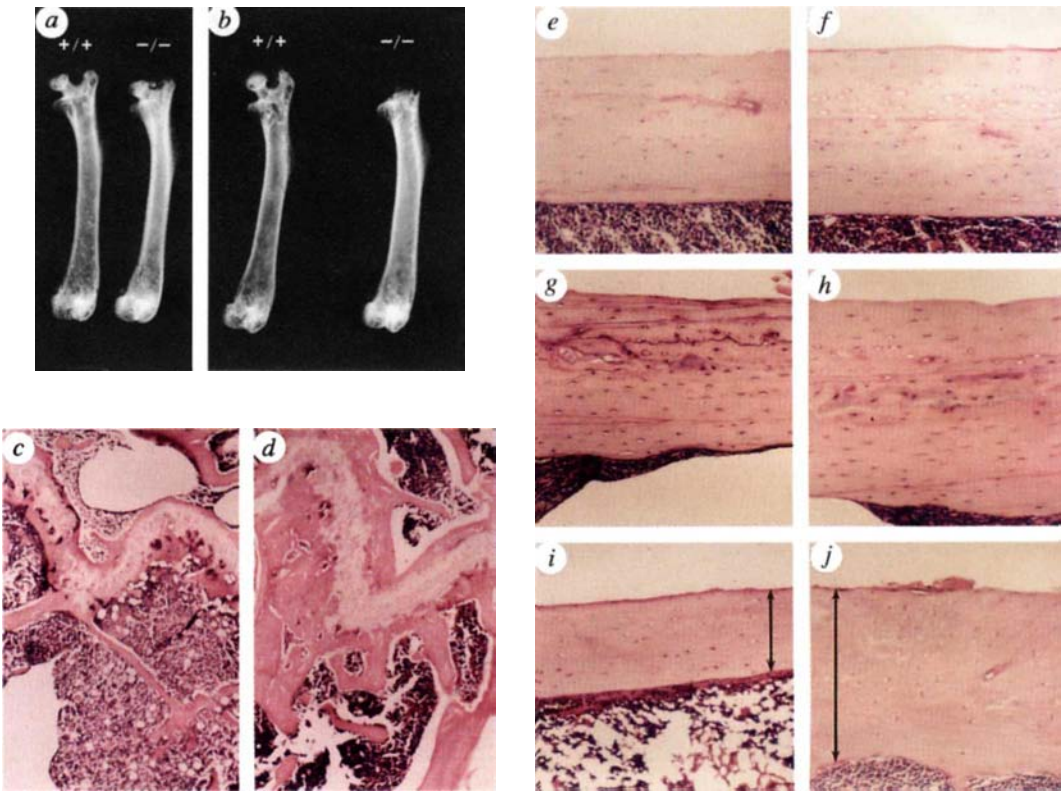
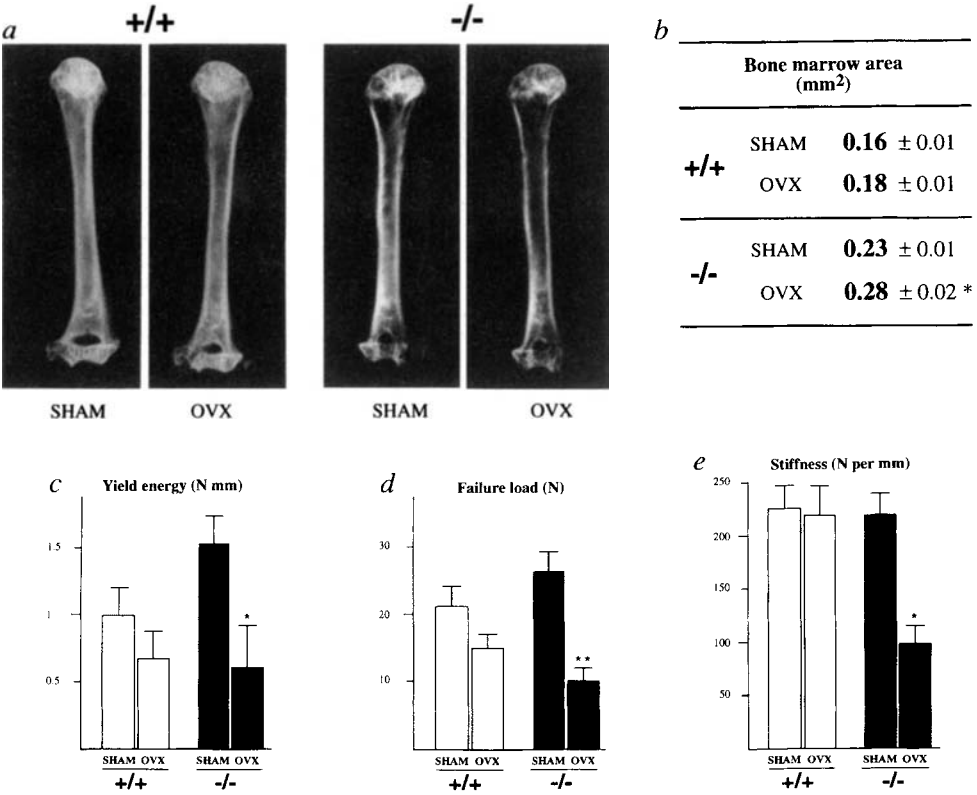


FIG. 4 *In vivo* analysis of bone resorption in *osc^{m1}/osc^{m1}* mice. 5-month-old wild-type (+/+) and homozygous *osc^{m1}/osc^{m1}* (-/-) mice were sham-operated (SHAM) or ovariectomized (OVX), and killed 28 days later. **a**, X-ray analysis of long bones (humerus) of SHAM (left) or OVX (right) 6-month-old animals. Note the lighter aspect of the bones of the OVX mice, indicating a loss of bone mass. **b**, Histomorphometric measurements of the bone-marrow cavity area, which is significantly larger in OVX *osc^{m1}/osc^{m1}* mice (Student's *t*-test). **c-e**, Biomechanical analysis of the quality of the femora. Yield energy is an indicator of ductility of bone specimens. Failure load is a measure of the strength of the bone, and stiffness is an indicator of the elasticity of the bone⁸. The decreases in yield energy, failure load, and stiffness in OVX animals indicate a bone fragility similar to that in osteoporosis. **P* < 0.05, ***P* < 0.001, *n* = 6.

METHODS. Femora were tested to failure by four-point bending on an MTS servo-hydraulic testing machine at a constant displacement rate of 0.5 mm s⁻¹ (ref. 8). Stiffness was calculated as the slope of the linear portion of the load-displacement curve, and yield energy was determined as the area under the load-displacement curve. Statistical significance was determined by one-way ANOVA with Tukey's post-hoc test for group comparison.



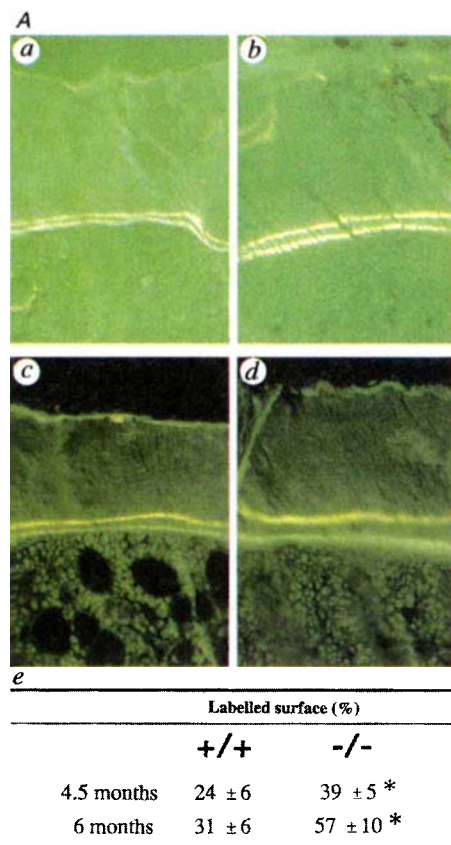
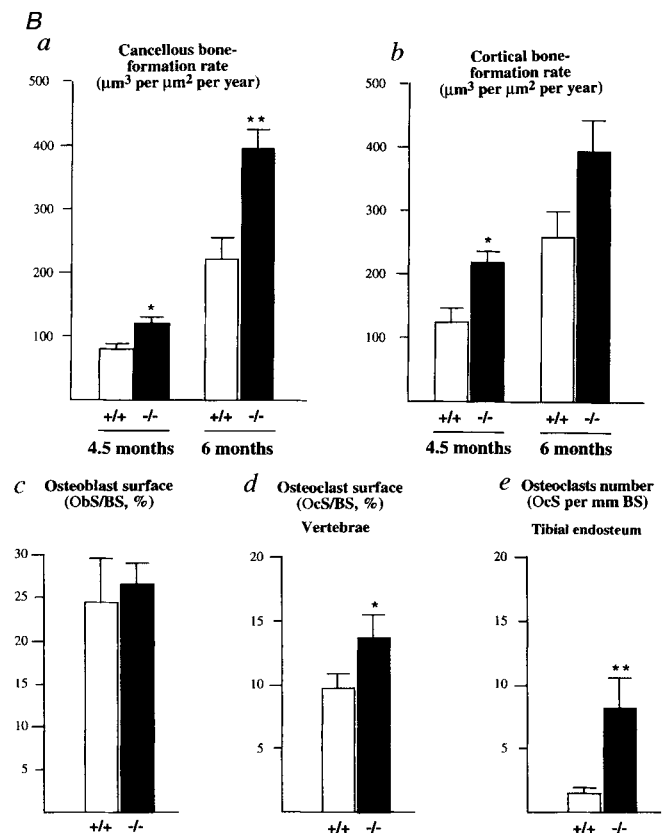


FIG. 3 *In vivo* analysis of bone formation in *osc^{m1}/osc^{m1}* mice. *a*, Tetracycline/calcein double-labelling analysis in *osc^{m1}/osc^{m1}* mice. *a-d*, Fluorescent micrographs of the two labelled mineralization fronts in representative sections of the mid-diaphysis of the femora of wild-type (*a*, *c*) and *osc^{m1}/osc^{m1}* mice (*b*, *d*) of 4.5 (*a*, *b*) and 6 months old (*c*, *d*). Note the increase in cortical thickness and in the distance between the two labelled areas, as well as the wider aspect of the labelled areas in the mutant animals, reflecting the increase in bone-matrix deposition rate. *e*, Quantification of the percentage of labelled surface in wild-type (+/+) and mutant (-/-) littermates. *B*, Static and dynamic histomorphometric analysis of vertebrae and femora of wild-type (+/+) and homozygous *osc^{m1}/osc^{m1}* (-/-) mice. Measurement of bone-formation rates (*a*, *b*) and cell surfaces (*c-e*) in wild-type and *osc^{m1}/osc^{m1}* mice of 4.5 and/or 6 months old. Bars represent means ± s.e.m. Asterisks indicate a statistically significant



difference between wild-type and *osc^{m1}/osc^{m1}* mice groups (* $P < 0.05$, ** $P < 0.005$). For 4.5 months, $n = 4$; for 6 months, $n = 7$. **METHODS.** Double labelling has been described²². Tetracycline injection (25 mg per kg body weight) was followed by the same dose of calcein 10 days later. Animals were killed 2 days after the second injection, and their bones were dissected and fixed in PBS-buffered formaldehyde (pH 7.1). After embedding in methylmethacrylate and sectioning at 6 μm , a blind analysis of the unstained samples was conducted under fluorescent light. Histomorphometric parameters follow the recommended nomenclature¹⁰. Osteoblast surface was measured according to published protocols⁹ with the Bioquant Image Analysis System (R&M Biometrics). Osteoclast surface was measured as the surface of tartrate-resistant acid-phosphatase-positive cells²³⁻²⁵. Statistical differences between groups were assessed by Student's *t*-test.

littermates (Fig. 3A), indicating that the lack of osteocalcin leads to an increase in bone formation. Likewise, the cancellous and cortical bone-formation rates were significantly increased in the long bones and vertebrae of *osc^{m1}/osc^{m1}* mice (Fig. 3B, *a* and *b*). Remarkably, considering the increase in bone-formation rates, the osteoblast surface, which is a reliable indicator of the number of osteoblasts^{9,10}, was not increased in the mutant animals (Fig. 3B, *c*).

Another important finding of the histomorphometry analysis was the increase in osteoclast number and surface in the bones of *osc^{m1}/osc^{m1}* mice (Fig. 3B, *d* and *e*). This raised the possibility that the osteoclasts were functioning poorly in mutant mice. To test the function of the osteoclasts *in vivo*, 5-month-old wild-type and mutant mice were ovariectomized. Ovariectomy, and the oestrogen depletion it creates, causes a rapid increase in bone resorption which ultimately leads to osteoporosis^{11,12}. X-ray analysis one month after ovariectomy showed the expected slight decrease in bone density in wild-type mice¹³. This decrease was greater in *osc^{m1}/osc^{m1}* animals (Fig. 4a). Histomorphometric analysis showed an increase in bone-marrow area, a good indicator of osteoclast function^{10,13}, in both wild-type and mutant animals after ovariect-

omy (Fig. 4b). Biomechanical studies showed that the long bones of ovariectomized *osc^{m1}/osc^{m1}* mice were weaker than the bones of sham-operated animals (Fig. 4c-e). These results demonstrate that bone resorption occurs normally in *osc^{m1}/osc^{m1}* mice. Consistent with the increase in osteoclast surface in mutant mice before ovariectomy, these animals may develop a more severe osteoporosis than their wild-type littermates after oestrogen depletion.

To determine whether osteocalcin plays a role in bone mineralization, von Kossa staining was performed, and both mineral apposition rates and bone mineral contents were measured. By these criteria, wild-type and mutant bones were indistinguishable (data not shown), indicating that the absence of osteocalcin did not affect bone mineralization.

Thus, osteocalcin normally functions to limit bone formation without impairing bone resorption or mineralization. The molecular mechanism by which osteocalcin controls bone matrix deposition is unknown. The increase in bone formation rates occurs without a comparable increase in osteoblast surface, indicating that each osteoblast is laying down more matrix. This suggests that osteocalcin, like other members of the family of

proteins to which it belongs^{14,15}, may bind to a specific, yet to be identified, receptor to fulfil its function. The identification of osteocalcin as a negative regulator of bone formation suggests that an antagonist of osteocalcin synthesis or function, in conjunction with oestrogen replacement therapy, may be of some therapeutic use. □

Received 19 March; accepted 28 May 1996.

1. Soriano, P., Montgomery, C., Geske, R. & Bradley, A. *Cell* **64**, 693–702 (1991).
2. Wang, Z.-Q. et al. *Nature* **360**, 741–745 (1992).
3. Grigoriadis, A. E. et al. *Science* **266**, 443–448 (1994).
4. Hauschka, P., Lian, J., Cole, D. & Gundersen, C. *Physiol. Rev.* **69**, 990–1047 (1989).
5. Weinreb, M., Shinar, D. & Rodan, G. A. *J. Bone Miner. Res.* **5**, 831–842 (1990).
6. Boivin, G. et al. *Virchows Arch. A. Path. Anat.* **417**, 505–512 (1990).
7. Desbois, C., Hogue, D. A. & Karsenty, G. *J. Biol. Chem.* **269**, 1183–1190 (1994).
8. Bonadio, F. et al. *Proc. natn. Acad. Sci. U.S.A.* **87**, 7145–7149 (1990).
9. Poli, V. et al. *EMBO J.* **13**, 1189–1196 (1994).
10. Parfitt, A. M. et al. *J. Bone Miner. Res.* **2**, 595–610 (1987).
11. Parfitt, A. M. et al. *J. clin. Invest.* **72**, 1396–1409 (1983).
12. Parfitt, A. M., Riggs, B. L. & Melton, L. J. *Osteoporosis: Etiology, Diagnosis and Management* (eds Parfitt, A. M., Riggs, B. L. & Melton, L. J.) 501 (Raven, New York, 1988).
13. Bain, S. D., Bailey, M. C., Celino, D. L., Lantry, M. M. & Edwards, M. W. *J. Bone Miner. Res.* **8**, 435–442 (1993).
14. Stitt, T. N. et al. *Cell* **80**, 661–670 (1995).
15. Coughlin, S. *Thromb. Haemost.* **70**, 184–187 (1993).
16. McMahon, A. P. & Bradley, A. *Cell* **62**, 1073–1085 (1990).
17. Luo, G., D'Souza, R., Hogue, D. & Karsenty, G. *J. Bone Miner. Res.* **10**, 325–334 (1995).
18. Oldberg, A., Franzen, A. & Heinegard, D. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8819–8823 (1986).
19. Ramirez-Solis, R., Davis, A. & Bradley, A. *Meth. Enzym.* **225**, 855–878 (1993).
20. Bradley, A. *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach* (ed. Robinson, E. J.) 113–151 (IRL, Oxford, 1987).
21. Gundersen, C., Hauschka, P., Lian, J., Gallop, P. M. *Meth. Enzym.* **107**, 516–566 (1984).
22. Vignery, A. & Baron, R. *Anat. Rec.* **196**, 191–200 (1980).
23. Andersson, G. N. & Marks, S. J. *J. Histochem. Cytochem.* **37**, 115–117 (1989).
24. Boyce, B. F., Yoneda, T., Lowe, C., Soriano, P. & Mundy, G. R. *J. clin. Invest.* **90**, 1622–1627 (1992).
25. Jilka, R. L. et al. *Science* **257**, 88–91 (1992).

ACKNOWLEDGEMENTS. We thank K. Hagihara for the western blot, E. Loyer and I. Schwartz for X-ray analysis, and K.-F. Tseng and A. Farias for help with the project. G.K. thanks R. Baron and A. Nanci for advice and H. Bellen, L. Etkin, R. Gagel, S. Goldring, W. Klein and G. Mundy for critically reading the manuscript. This work was supported by grants from the NIH and the March of Dimes Foundation. A.B. is an investigator with the Howard Hughes Medical Institute.

CORRESPONDENCE and requests for materials should be addressed to G.K. (e-mail: gkarsenty@molgen.mda.uth.tmc.edu).

Induction of a specific muscle cell type by a hedgehog-like protein in zebrafish

Peter D. Currie & Phillip W. Ingham

Molecular Embryology Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

THE notochord plays a central role in vertebrate development, acting as a signalling source that patterns the neural tube and somites^{1–4}. In *in vitro* assays, the secreted protein Sonic hedgehog mimics the inducing effects of notochord on both presomitic mesoderm and neural plate explants of amniote embryos, suggesting that both patterning activities of the notochord may be mediated by this protein *in vivo*^{5–8}. In zebrafish, however, mutants with disrupted notochord development lack a specific muscle cell type, the muscle pioneers, although they retain the ability to induce neural differentiation, raising the possibility that neural tube and somite patterning may be mediated by distinct signals^{9,10}. Here we describe a new member of the *hedgehog* family, *echidna hedgehog*, that is expressed exclusively in the notochord and has the ability to rescue the differentiation of muscle pioneer cells in mutants with no notochord. Moreover, we show that a combination of ectopic *echidna hedgehog* and *sonic hedgehog* expression induces supernumerary muscle pioneers in wild-type

embryos, suggesting that both signals act sequentially to pattern the developing somites.

Embryos homozygous for the zebrafish mutations *no tail* (*ntl*) and *floating head* (*flh*) lack differentiated notochords and exhibit concomitant somite defects^{9,10}. In both cases, somites are irregularly shaped and lack a horizontal myoseptum, a structure of the dorsoventral midline of the myotome. They also lack a specialized group of muscle cells, the muscle pioneers (MPs), which are amongst the earliest differentiating and migrating cells of the myotome^{9–12}. MPs can easily be distinguished from other muscle cells as they express *engrailed* (*en*)¹¹. In wild-type fish, between two and six MPs form directly apposed to the notochord in each somite, aligned at the level of the horizontal myoseptum¹¹.

Both the *ntl* and *flh* genes encode transcription factors whose expression is principally confined to the axial mesoderm that gives rise to the notochord^{9,10}. Thus the somite defects seen in each mutant are secondary consequences of their effects on notochord differentiation, reflecting the role of the notochord in somite patterning. Importantly, however, expression of the putative notochord-derived signal, *sonic hedgehog* (*shh*), is retained during early stages of development in *flh* and *ntl* embryos and, in line with this observation, induction of floorplate differentiation in the ventral neural tube occurs, to varying extents in both mutants^{10,13}. Taken together these findings suggest that Shh activity is not sufficient for MP induction and imply the existence of some other notochord-derived MP-inducing activity.

We identified one member of the hedgehog (*hh*) gene family cloned in zebrafish¹³, *echidna hedgehog* (*ehh*), which is expressed exclusively within cells of the presumptive notochord. The sequence of the *ehh* open reading frame reveals a high degree of sequence identity with other Hedgehog proteins (Fig. 1a). All amino-acid motifs implicated in Shh function are conserved in the Ehh protein, and like *shh*, *ehh* can activate the *hh* signalling pathway in *Drosophila* when misexpressed in imaginal discs (unpublished observations). In the developing zebrafish embryo, expression of *ehh* is first detectable somewhat later than is expression of *shh*, at the mid-gastrula stage in cells of the axial midline¹³ (data not shown). Expression spreads along the midline as the axial mesoderm extends towards the animal pole (Fig. 2a), and by early somitogenesis its anterior and dorsoventral limits can be seen to coincide with that of the notochord (Fig. 2a–c). As somitogenesis continues, expression diminishes in a rostral-to-caudal progression along the length of the notochord, becoming confined to the tail notochord by the 26-somite stage (Fig. 2e) and disappearing completely by the end of somitogenesis. By comparison, *shh* expression is found within cells of the neural tube and notochord during somitogenesis and this expression is maintained after somitogenesis is completed (Fig. 2d, f).

Unlike *shh*, expression of *ehh* cannot be detected at any stage in either *ntl* or *flh* homozygous embryos (Fig. 2g–j, and data not shown). Thus absence of MPs correlates with absence of *ehh* expression, suggesting that *ehh* may be required for MP induction. To test this, we assayed the activity of *ehh* using the same over-expression methods used previously to analyse *shh* function¹³. Injection of *shh* sense messenger RNA into 1–2-cell-stage embryos produces a number of morphological abnormalities, notably a disruption of eye development, but has no obvious morphological effect on the development of the somites^{13–15} (80%, *n* = 96; data not shown). Similar effects are induced by ectopic expression of another hedgehog-family gene, *tiggy-winkle hedgehog* (*nwhh*) (67%, *n* = 45)¹⁴, that is also expressed along the midline of the developing embryo¹⁴. By contrast, *ehh* sense mRNA fails to produce any morphologically detectable phenotype when injected under identical conditions (*n* = 162), or even when injected at 5 times higher concentrations (*n* = 45). To test the possibility that this lack of effect reflects an inefficient processing of the Ehh protein, we generated a truncated mRNA that encodes only the predicted mature amino-terminal fragment of Ehh. Injection of this mRNA under identical conditions similarly resulted in no morphological defects (*n* = 53). However, when

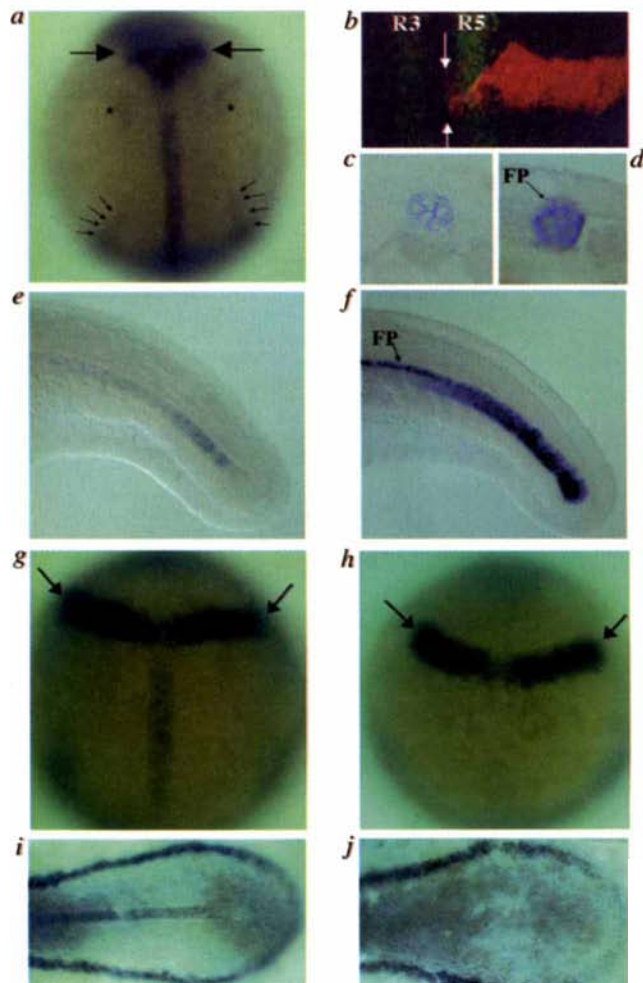


FIG. 2 Expression pattern of *ehf*. *a*, One-somite embryo hybridized simultaneously with antisense mRNA to *pax2* and *ehf*. At this developmental stage *pax2* mRNA is localized to the midbrain–hindbrain junction (large arrows), otic placodes (asterisks), and in the lateral-plate mesoderm of the nephritic primordia (small arrows)²². *ehf* expression extends rostrally to a position level with the otic placode, the morphological limit of the notochord, within rhombomere 4. *b*, *ehf* expression at 2–3 somites extends to the morphological limit of the notochord, within rhombomere 4. A confocal image of the expression of *ehf* (red), and *krox 20* (green) demonstrates that *ehf* expression extends between the two *krox 20* strips which are expressed in rhombomeres 3 (R3) and 5 (R5)²³. *c*, A cross-section through the axial midline at the level of the trunk notochord at 4–5 somites reveals expression only within the cells of the notochord and not within cells of the neural tube. *d*, An embryo at a similar development stage, hybridized with *shh* antisense mRNA, reveals expression both in notochord and floorplate (FP) cells. *e*, Late somitogenesis (26 somites) expression of *ehf* is limited to a few of the most caudal cells of the tail notochord but not the tail bud. *f*, At the same stage, expression of *shh* persists in tail notochord cells and in cells of the floor plate (FP). *g*, *i*, Expression of *ehf* typical of the wild type is absent in 25% of the siblings derived from *ntl*/+ (*h*) and *flh*/+ (*j*) parents. *g*–*j*, Embryos hybridized simultaneously with antisense probes to *ehf* and *pax2* as a hybridization control. Embryos are at 90–100% epiboly and *pax2* message marks the midbrain–hindbrain junction at this stage (large arrows) (*g*, *h*). *i*, *j*, Embryos at 1–2 somites. *pax2* message is expressed laterally in the nephritic primordia in this more caudal view. In *a*, *g* and *h*, anterior is to the top of the page. In *b*, *e*, *f*, *i* and *j*, anterior is to the left. In *c* and *d*, dorsal is to the top.

METHODS. *In situ* hybridization with diogoxigenin incorporated antisense probes used standard techniques²⁴. Differential labelling was accomplished by using fast red tablets (Boehringer) and enzyme-linked fluorescence (Molecular probes) following the manufacturers instructions and standard techniques²⁴. Specimens were analysed using a BioRad MRC 1000 confocal microscope. Embryos in panels *i* and *j* have been mounted flat with yolk removed. Fish embryo raising and staging was as described^{25,26}.

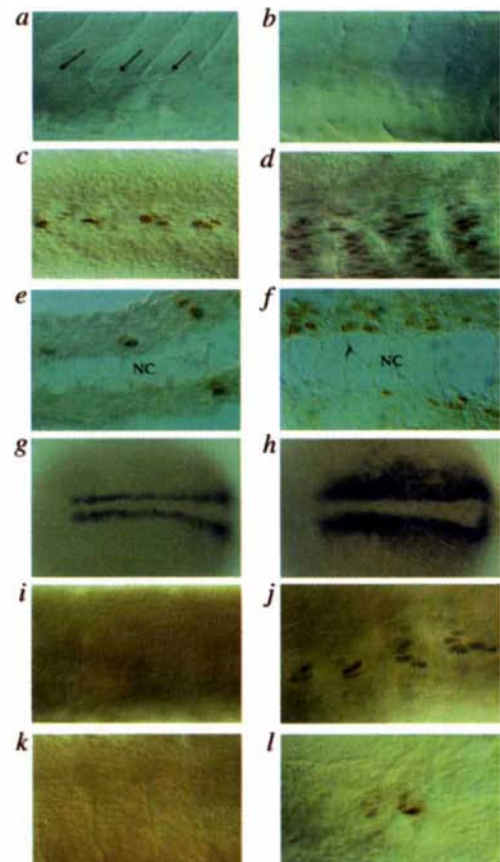


FIG. 3 Phenotypes of embryos injected with *ehf* and/or *shh* sense mRNA. Embryos are at the 26-somite stage and viewed with DIC optics unless otherwise stated. Anterior is to the left and dorsal to the top unless otherwise stated. *a*–*f*, *i*–*l*, These show four somites within each field. Images of wild-type (*a*) and co-injected (*b*) embryos visualized at the level of the somite. The somites of doubly injected embryos appear disorganized and lack a horizontal myoseptum, which is evident in wild-type embryos (arrows). Injected embryos (*d*) show a 10–20-fold increase in the number of MPs compared with uninjected embryos (*c*). *c*, *e*, Wild-type somites in a lateral view and transverse oblique section reveal 2–6 cells per somite. *d*, *f*, Similar views of co-injected embryos reveal ectopic muscle pioneers at all levels of the somite but concentrated more ventrally. *In situ* hybridization of wild-type (*g*) or co-injected embryos (*h*) at bud stage with an antisense probe to the *myoD* gene. Dorsal view with anterior to the left. Ectopic induction of *myoD* up to 20–30 cell diameters from the normal expression domain can be seen in co-injected embryos. *i*, *k*, *flh* and *ntl* mutant embryos lack muscle pioneers. Injection of *ntl* (*j*) and *flh* (*l*) homozygous embryos with *ehf* sense mRNA rescues the differentiation of MPs. Rescue typically occurs across 4–5 somites in *ntl* embryos and 1–2 somites in *flh* mutant embryos. Rescue occurs more predominantly within anterior somites.

METHODS. Injections into 2- or 4-cell-stage zebrafish embryos were as described previously¹³. Initially a dilution series of message injection in distilled H₂O was performed for single and double injections. Concentration of 0.01, 0.1, 0.5 and 1 $\mu\text{g}\mu\text{l}^{-1}$ were injected. The 1 $\mu\text{g}\mu\text{l}^{-1}$ concentration produced a high level of lethality and non specific developmental defects for both singly and doubly injected embryos, and was not used further. There were no morphological defects for any injections at 0.01 $\mu\text{g}\mu\text{l}^{-1}$. The 0.1 and 0.5 $\mu\text{g}\mu\text{l}^{-1}$ concentrations produced similar effects, and 0.1 $\mu\text{g}\mu\text{l}^{-1}$ was chosen for the experiments shown. To control for amounts of injected message the same needle was used for both single and double message injections, with the needle being rinsed with H₂O before each loading. Whole-mount immunohistochemistry used the monoclonal antibody 4D9, which was raised against the *Drosophila invected* gene product, and recognizes *En*-specific antigens in many species by standard methods^{11,25}. *In situ* hybridization was with the *myoD* gene antisense RNA using standard techniques²⁴.

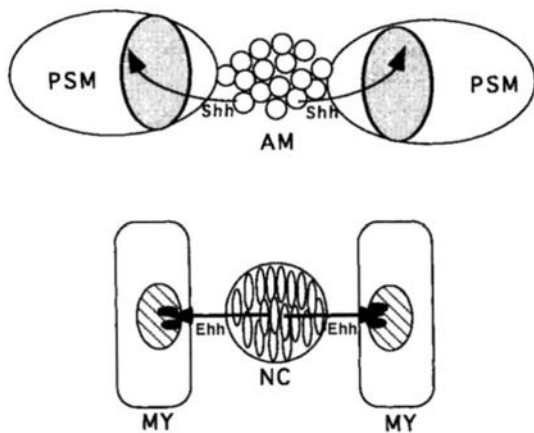


FIG. 4 Model for the coordinated action of Shh and Ehh in the developing zebrafish somite. **a**, Secretion of Shh from within the axial mesoderm (AM) acts to induce or recruit cells of the presomitic mesoderm (PSM), to a myotomal lineage, (shaded region) **b**, As somitogenesis proceeds, Ehh is secreted by the notochord (NC), which acts to pattern a specific subset (hatched region) of the myotome (MY) as exemplified by the differentiation of muscle pioneer cells (in black).

embryos (*flh*, 53%, $n = 42$; *ntl*, 58%, $n = 68$) (compare Fig. 3i, k with j, l). Notably, the number of cells rescued in *ntl* embryos tends to be higher than in *flh* embryos, possibly reflecting a more robust induction of MP precursors by the more extensive expression of *shh* in the former^{9,10}. By contrast, MPs were never observed in embryos homozygous for either mutant after injection of *shh* mRNA alone (*ntl*, $n = 36$; *flh*, $n = 28$).

Our findings imply a role for different Hh family proteins at different stages of a multi-step process leading to the specification of a distinct muscle lineage. Interestingly, recent mutant screens of the zebrafish genome have identified several genes required for different steps in this pathway¹⁷. To what extent these reflect differences in the components of the pathways that transduce the two signals remains to be seen. The specificity of Ehh activity does, however, imply the existence of distinct receptors for different Hh family proteins. Although the N-terminal domains of Ehh and Shh show a high degree of sequence identity, there are a number of non-conservative changes that may provide the basis for this specificity. Finally we note that MPs seem to be unique to teleosts and orthologues of *ehh* have thus far not been found in any other vertebrate classes. However, there may well be other cell types in other vertebrate species that are similarly induced by specific Hh-family proteins. □

Received 31 January; accepted 14 June 1996.

1. van Straaten, H. W. M. et al. *Development* **107**, 793–803 (1989).
2. Yamada, T. et al. *Cell* **73**, 673–686 (1993).
3. Yamada, T. et al. *Cell* **64**, 635–647 (1991).
4. Buffinger, N. & Stockdale, F. E. *Development* **120**, 1443–1452 (1994).
5. Munsterberg, A. E. et al. *Genes Dev.* **9**, 2911–2922 (1995).
6. Marti, E. et al. *Nature* **375**, 322–325 (1995).
7. Roelink, H. et al. *Cell* **81**, 445–455 (1995).
8. Johnson, R. L. et al. *Cell* **79**, 1165–1173 (1994).
9. Halpern, M. E. et al. *Cell* **75**, 99–111 (1993).
10. Talbot, W. S. et al. *Nature* **378**, 150–157 (1995).
11. Hatta, K. et al. *Development* **112**, 821–832 (1991).
12. Felsenfeld, A. L., Curry, M. & Kimmel, C. B. *Dev. Biol.* **184**, 23–30 (1991).
13. Krauss, S., Concordet, J. P. & Ingham, P. W. *Cell* **75**, 1431–1444 (1993).
14. Ekker, S. C. et al. *Curr. Biol.* **5**, 944–955 (1995).
15. MacDonald, R. et al. *Development* **121**, 3267–3278 (1995).
16. Weinberg, E. S. et al. *Development* **122**, 271–280 (1996).

17. van Eeden, F. J. M. et al. *Development* (in the press).
18. Burncroft, D. A., Takada, R. & McMahon, A. P. *Molec. cell. Biol.* **15**, 2294–2303 (1995).
19. Porter, J. A. et al. *Nature* **374**, 363–366 (1995).
20. Hall, T. M. T. et al. *Nature* **378**, 212–216 (1995).
21. Lee, J. J. et al. *Science* **266**, 1528–1537 (1994).
22. Krauss, S. et al. *Development* **113**, 1193–1206 (1991).
23. Oxtoby, E. & Jowett, T. *Nucleic Acids Res.* **21**, 1087–1095 (1993).
24. Jowett, T. & Lettice, L. *Trends Genet.* **10**, 73–74 (1994).
25. Westerfield, M. *The zebrafish book—A guide for laboratory use of zebrafish (Brachydanio rerio)* (Univ. Oregon Press, 1995).
26. Kimmel, C. B. et al. *Dev. Dynamics* **203**, 253–310 (1995).

ACKNOWLEDGEMENTS. We thank T. F. Schilling for extensive technical advice and comments on the manuscript and T. Jowett for technical advice. This research was supported by the ICRF and HFSP grant to P.W.I.

CORRESPONDENCE and requests for materials should be addressed to P.W.I. (e-mail: ingham@icrf.icnet.uk).

A non-muscle myosin required for embryonic polarity in *Caenorhabditis elegans*

Su Guo & Kenneth J. Kemphues

Section of Genetics and Development, 101 Biotechnology Building, Cornell University, Ithaca, New York 14853, USA

DAUGHTER cells with distinct fates can arise through intrinsically asymmetrical divisions¹. Before such divisions, factors crucial for determining cell fates become asymmetrically localized in the mother cell^{2,3}. In *Caenorhabditis elegans*, PAR proteins are required for the early asymmetrical divisions that establish embryonic polarity^{4–8}, and are asymmetrically localized in early blastomeres^{9,10}, although the mechanism of their distribution is not known. Here we report the identification in *C. elegans* of a non-muscle myosin II heavy chain (designated NMY-2) by means of its interaction with the PAR-1 protein, a putative Ser/Thr protein kinase. Furthermore, injections of *nmy-2* antisense RNA into ovaries of adult worms cause embryonic partitioning defects and lead to mislocalization of PAR proteins. We therefore conclude that NMY-2 is required for establishing cellular polarity in *C. elegans* embryos.

The *par-1* gene in *C. elegans* is required for proper intracellular asymmetry, and encodes a protein that shares two domains with a subfamily of protein kinases: the putative serine/threonine kinase domain, and a domain of unknown function at the carboxy terminus⁹. The striking conservation of the C-terminal domain led us to propose that it may be important for PAR-1 function.

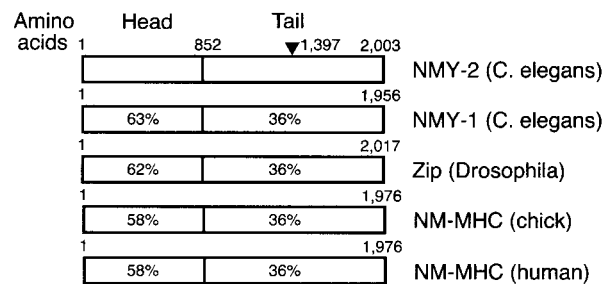
We investigated whether this domain is involved in protein–protein interactions by searching for proteins that bound to this domain by interaction cloning¹¹. After screening $\sim 5 \times 10^5$ clones from a *C. elegans* complementary DNA expression library, we isolated three clones that expressed proteins that bound to the C terminus of PAR-1. The three cDNA inserts were sequenced and found to be independent partial cDNAs of a single gene encoding a non-muscle myosin II heavy chain, which we named NMY-2 (Fig. 1). The coding regions of the three cDNAs overlap only for the C-terminal 607 amino acids, suggesting that PAR-1 is binding to the tail region of NMY-2.

We next determined that the PAR-1 C-terminal domain could interact with the NMY-2 tail region in solution (Fig. 2a), and that PAR-1 and NMY-2 could associate in whole worm extracts (Fig. 2b, c). Immunostaining of early embryos with affinity-purified anti-NMY-2 antibody shows that NMY-2, like PAR-1, is found at the periphery of early embryos (Fig. 3m). Taken together, these results suggest that PAR-1 and NMY-2 can associate physically in embryos.

Because PAR-1 and NMY-2 can interact *in vivo*, we investigated whether NMY-2, like PAR-1, is involved in establishing

FIG. 1 Comparison of NMY-2 and other non-muscle myosins^{21,24} (C. Newbern, J. Baker and D. Kiehart, personal communication; Y. Kohara, personal communication). The proteins share 45–50% amino-acid identity throughout, but a higher degree of conservation in the globular head domain than in the rod-like tail. The percentage of amino-acid identity in the head and tail regions is indicated. NMY-1 shares about 50% amino-acid identity with all of the molecules shown. The arrow indicates the start of the sequence shared by the cDNAs encoding NMY-2 fragments that interact with PAR-1.

METHODS. A *par-1* cDNA fragment encoding amino acids 1,020–1,192 was cloned into pGEX-2T (128/129) (M. Blañar). A fusion protein containing a heart muscle kinase (HMK) phosphorylation site, glutathione transferase and the PAR-1 C-terminal domain was expressed in *Escherichia coli* BL21 cells, and purified using glutathione-coupled agarose beads. The fusion protein was labelled using [γ -³²P]ATP and bovine HMK (Sigma), and used to screen a mixed-stage *C. elegans* λ gt11 cDNA expression library²⁵, as described¹¹. The cDNA inserts of positive phage plaques were amplified



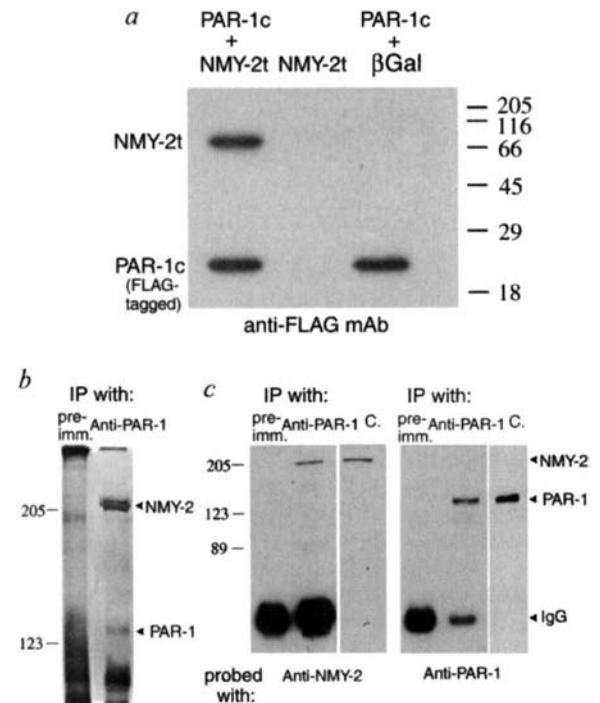
by polymerase chain reaction (PCR) using flanking primers in the vector, cloned into TA cloning vectors (Invitrogen) and sequenced. The 5' end of the *nmy-2* transcript was obtained by rapid amplification of cloned ends (RACE)-PCR (Clontech) and sequenced.

asymmetry. It seemed likely that null mutations in a non-muscle myosin heavy-chain gene would produce lethal or sterile phenotypes¹², so we used antisense RNA injection⁹ to disrupt endogenous *nmy-2* expression in the ovary; this allowed us to assess the role of maternally provided NMY-2 in early embryos. Injections of *in vitro*-transcribed antisense RNA into adult *C. elegans* ovaries has been used quite successfully to produce gene-specific loss-of-function phenotypes for several maternally expressed, embryonically required proteins^{9,13}. By an unknown mechanism, injections of *in vitro*-transcribed sense RNAs also produce gene-specific loss-of-function phenotypes^{9,13}. Wild-type worms injected with antisense or sense RNA transcribed from a 2.6-kilobase partial cDNA

encoding the 607 C-terminal amino acids of NMY-2 showed embryonic lethality that increased with time after injection (Table 1). Increased lethality correlated with a striking, time-dependent decrease in protein detected by immunofluorescence using anti-NMY-2 antibodies (Fig. 3n). This suggests that injections of *nmy-2* antisense RNA into the ovary result in progressive depletion of NMY-2 protein among embryos from the injected mother. Because another gene, *nmy-1*, identified by the *C. elegans* Genome Sequencing Project, also appears to encode a non-muscle myosin, it was possible that the antisense injection was affecting expression of both genes. This does not seem to be the case as injections of antisense RNA transcribed from a 3.2-kb

FIG. 2 Complex formation *in vitro* between the PAR-1 C-terminal domain (PAR-1c) and the tail portion of NMY-2 (NMY-2t) in solution, and co-immunoprecipitation of NMY-2 with PAR-1 from whole worm extracts. **a**, Autoradiogram of SDS-polyacrylamide gel electrophoresis (SDS-PAGE) showing results of immunoprecipitation of NMY-2t and PAR-1c made *in vitro* with anti-FLAG monoclonal antibody (mAb). Left, NMY-2t can be co-immunoprecipitated with the FLAG-tagged PAR-1c by anti-FLAG mAb. Middle (blank), results of a control immunoprecipitation in the absence of PAR-1c. Right, results of a control experiment with *in vitro*-translated PAR-1c and β -galactosidase. **b**, Silver-stained SDS-PAGE showing the results of immunoprecipitation of PAR-1 from worm extracts. Left, control immunoprecipitate (IP) with pre-immune serum; right, IP using anti-PAR-1. The difference in staining intensity between PAR-1 and NMY-2 could be due to differences in sensitivity to silver stain, or mean that each PAR-1 molecule is complexed with multiple NMY-2 molecules. **c**, Each series of three lanes shows western blots of: left, IP with pre-immune serum; middle, IP with anti-PAR-1; right, a sample of the extract before IP. The three lanes on the left were probed with anti-NMY-2, those on the right with anti-PAR-1. Rough quantification based on ECL signals suggests that ~30% of total PAR-1 and ~5% of total NMY-2 in the supernatant were immunoprecipitated.

METHODS. For *in vitro* co-immunoprecipitation, the cDNA fragment encoding the PAR-1 C-terminal domain (amino acids 1,020–1,192) was cloned into pAR1 (59/60) (M. Blañar), which expressed a FLAG epitope tag. The cDNA fragment encoding the C-terminal 607 amino acids of NMY-2 (1,397–2,003) was cloned into pET16b (Novagen). The two plasmids were transcribed with T7 RNA polymerase using Stratagene *in vitro* capping kit; RNA encoding β -gal was transcribed from the vector pJK350' using SP6 RNA polymerase. The mRNAs were translated separately using a rabbit reticulocyte lysate system (Promega) with [³⁵S] methionine. The translation products were mixed and incubated at room temperature for 45 min, before 10-fold dilution with phosphate-buffered saline (PBS) containing 0.1% NP-40, and precleared by incubating with protein A-CL4B beads (Sigma) for 30 min at 4 °C. The anti-FLAG monoclonal antibody M2 (IBI/Kodak) was added to the supernatant after preclearing, and the mixture incubated on ice for 1.5 h, followed by adding protein A-CL4B beads and incubating for 30 min. Beads were pelleted, washed with PBS/0.1% NP-40 six times, and resuspended in SDS sample buffer, before analysis by SDS-PAGE. The gel was soaked in 1 M salicylic acid, pH 6, for 30 min to enhance the signal, dried and exposed to X-ray film. For co-immunoprecipitation from worm extracts, gravid hermaphrodites were washed off plates with water, then PBS, and lysis buffer (100 mM NaCl, 50 mM Tris.HCl, pH 8, 10% glycerol, 0.5% NP-40, with protease inhibitors: 10 mg ml⁻¹ PMSF, 1 mg ml⁻¹ leupeptin, pepstatin, aprotinin). Worms (1 ml) were resuspended in ~3-ml



lysis buffer. The sample was homogenized in a Dounce homogenizer: (3 sets of 5 strokes each with pestle B, 10 sets of 5 strokes each with pestle A, with 30 second incubation on ice between each set), then unbroken worms were pelleted by a brief spin in a bench-top centrifuge, and the homogenate was then centrifuged at 32,500g for 10 min. The supernatant was pre-cleared with protein A-CL4B beads, then the supernatant was incubated with antibody-coupled protein A-CL4B beads at 4 °C for 1 h with gentle agitation. Beads were washed six times with lysis buffer, boiled in SDS sample buffer for 5 min, and analysed by SDS-PAGE and western blot. Anti-NMY-2 antibodies were raised in rabbits against fusion proteins containing amino acids 440–736 of NMY-2, and affinity purified.

TABLE 1 Embryonic lethality caused by antisense and sense *nmy-2* RNA injection

RNA injected	Number of worms injected	Time after injection (h)	Embryonic lethality (%)*
Antisense	15	0–17	2 (457)
		18–24	52 (418)†
		>24	100 (135)‡
Sense	16	0–17	2 (594)
		18–24	25 (418)†
		>24	98 (363)‡

* The number in parentheses is the total number of embryos (average brood from an uninjected worm is about 300).

† Most of the surviving embryos were agametic, a phenotype seen in weak *par* mutations⁴.

‡ Injected worms cease egg production prematurely, leading to lower overall brood size.

partial cDNA encoding the C terminus of *nmy-1* failed to produce embryonic lethality. We conclude that *nmy-2* gene expression is required for embryogenesis.

To determine whether depleting NMY-2 caused defects in early embryonic divisions, we examined early embryos from injected mothers by time-lapse video recording. Most embryos dissected ~18–24 h after injection had Par-like phenotypes (Fig. 3), suggesting that *nmy-2*, like the *par* genes, is required for establishing polarity in *C. elegans* embryos.

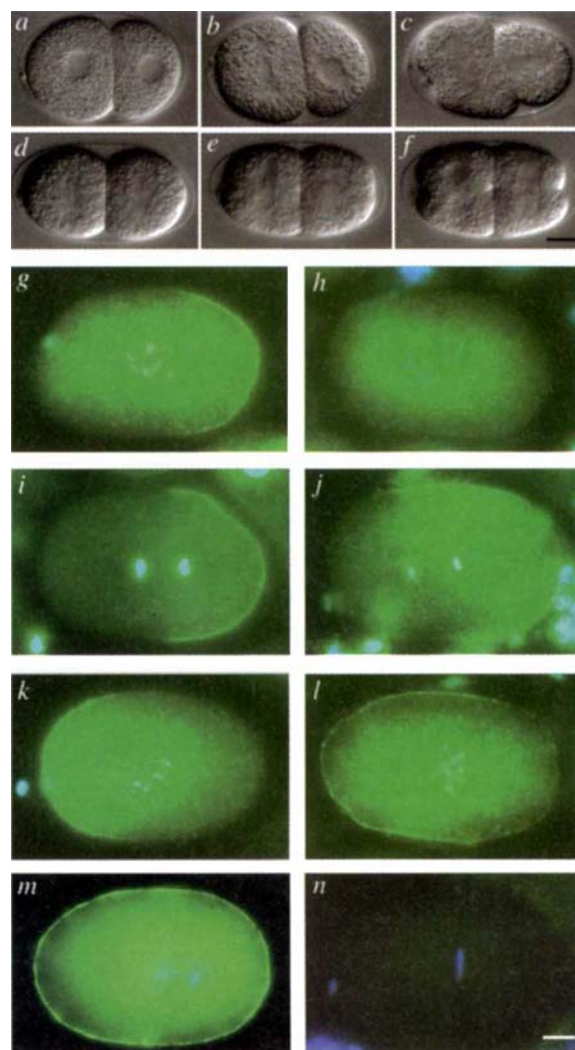
FIG. 3 NMY-2 is required for embryonic polarity and asymmetrical localization of PAR proteins. *a–c*, Nomarski images of wild-type embryos showing unequal first cleavage, asynchronous second divisions, and blastomere-specific spindle orientations in AB (left) and P1 (right)²⁶. *d–f*, Nomarski images of NMY-2 depleted embryos showing equal first cleavage, synchronous second divisions, and AB-like division orientation in P1 owing to the failure of centrosome-nuclear rotation. Scale bar, 10 μ m. *g–l*, Immunolocalization of PAR-1, PAR-2 and PAR-3 in wild-type (*g, i, k*) and NMY-2-depleted embryos (*h, j, l*). PAR-1 is asymmetrically localized to the posterior periphery in the wild-type zygote (*g*), but is not detectable at the periphery in the NMY-2-depleted embryo (*h*). PAR-2 is asymmetrically localized to the posterior cortex in the wild-type zygote (*i*), but fails to be restricted to the posterior in the NMY-2-depleted embryo, often with variably positioned patches of more intense staining as shown here (*j*); PAR-3 is asymmetrically localized to the anterior cortex in the wild-type zygote (*k*), but is detected around the entire cortex in the NMY-2-depleted embryo (*l*). *m, n*, Immunolocalization of NMY-2 in wild-type and NMY-2-depleted one-cell embryos. The embryo in *n* was within a mother fixed 24 h after injection. Scale bar, 7 μ m.

METHODS. The plasmid containing the fragment encoding the tail portion of NMY-2 was linearized with *KpnI*, and *in vitro* transcribed with T7 RNA polymerase to produce antisense RNA (*in vitro* capping kit, Stratagene). The antisense RNA was injected into the syncytial gonad of wild-type (N2) hermaphrodites, as previously described⁹. Injected animals were plated out at 20 °C. The early embryonic phenotypes were observed by cutting worms open in water 20–24 h after injection, and transferring the live early embryos to polylysine-coated slides for video microscopy. Most embryos *in utero* at this time would have been laid after 24 h postinjection, and would be expected to be inviable (see Table 1). Of 25 one-cell embryos sampled, 17 showed partitioning defects, and 8 were blocked in cytokinesis. To examine the PAR protein distributions, the *nmy-2* antisense RNA-injected worms were fixed 18–24 h after injection, and stained with PAR-1, PAR-2 and PAR-3 antibodies as described^{9,10} (L. Boyd *et al.*, manuscript submitted). A minimum of 40 injected worms were used for the staining of each antibody. The described distribution patterns were observed in: 25 of 32 (PAR-1), 24 of 30 (PAR-2), and 19 of 19 (PAR-3) one-cell embryos examined. To examine NMY-2 distribution, embryos were fixed using the same protocol used for PAR-1 staining⁹, and stained with affinity-purified rabbit polyclonal anti-NMY-2 antibody. Injected worms (15) were fixed 18, 22 and 24 h after antisense injection, with uninjected controls for comparison. Staining in embryos showed a progressive decrease in staining intensity.

This Par-like phenotype was not the only phenotype observed in the dissected embryos. Some embryos dissected 18–28 h after injection, and all embryos dissected after 28 h, failed to undergo cytokinesis, so NMY-2, as well as being required for embryonic polarity, is also involved in cytokinesis. The requirement for non-muscle myosins in cytokinesis has been previously reported (reviewed in refs 14,15).

To determine whether NMY-2 is required for the localization of PAR proteins, we examined the distribution of PAR protein in NMY-2-depleted embryos. In the wild type, PAR-1 and PAR-2 are localized to the posterior periphery of one-cell embryos⁹ (L. Boyd, S. Guo, D. Levitan, D. Stinchcomb and K. Kemphues, manuscript submitted) and PAR-3 is localized to the anterior periphery¹⁰ (Fig. 3*g, i, k*). In NMY-2-depleted embryos, all three proteins are mislocalized (Fig. 3*h, j, l*): PAR-1 is not detected at the cell periphery, and PAR-2 and PAR-3, although remaining peripheral, are no longer restricted to posterior and anterior domains. In contrast, NMY-2 distribution does not seem to be affected by mutations in the *par* genes (data not shown). Thus we conclude that NMY-2 is required for proper localization of all three PAR proteins.

In summary, we have identified NMY-2, a *C. elegans* non-muscle myosin heavy chain, by means of its binding to the PAR-1 protein, and shown that both proteins interact *in vitro* and *in vivo*. Antisense inhibition analyses and immunolocalization studies show that NMY-2 is required for the establishment of embryonic polarity and for asymmetrical localization of PAR-1, PAR-2 and PAR-3 proteins. Our results demonstrate that con-



ventional myosin is required for the establishment of embryonic polarity. Together with previous analyses implicating microfilaments in generating polarity in *C. elegans*^{16,17}, our results suggest that actomyosin-based motility is involved in polarizing the zygote.

The interaction between PAR-1 and myosin might directly mediate the asymmetrical localization of PAR-1. The observation that NMY-2 is required for PAR-1 localization is consistent with this hypothesis. However, the finding that NMY-2 is also required to localize PAR-2 and PAR-3 raises the possibility that the role of myosin in localizing PAR-1 might not involve direct binding to PAR-1. Previous analysis has shown that normal PAR-1 localization depends upon functional *par-2* and *par-3* genes, whereas localization of PAR-2 and PAR-3 is not dependent upon *par-1* (ref. 10); L. Boyd *et al.*, manuscript submitted). Therefore, although it is possible that NMY-2 can interact with all three PAR proteins to cause their asymmetrical localization, at present we cannot exclude the possibility that NMY-2 localizes PAR-1

protein indirectly, perhaps through its action on PAR-2 or PAR-3. It is possible, therefore, that the interaction between PAR-1 and NMY-2 contributes to PAR-1 or NMY-2 function in some way other than localization of the PAR-1 protein. For example, PAR-1 might modify NMY-2, perhaps by phosphorylation, to allow localization of the germline-specific P granules to the posterior of the embryo. Experiments specifically blocking the binding of PAR-1 and NMY-2 *in vivo* are needed to determine the significance of this interaction.

Both the actin cytoskeleton and kinases are known to be involved in establishing cell polarity in yeast and mammalian epithelial cells¹⁸. Our results, combined with analysis showing that conventional myosin heavy chain contributes to cell polarity in *Dictyostelium*¹⁴, and the recent demonstration that an unconventional myosin is involved in asymmetrical cell division in yeast^{19,20}, suggest that the actin-based motor myosin might be widely used in generating cellular asymmetry. □

Received 4 March; accepted 6 June 1996.

- Horvitz, H. R. & Herskowitz, I. *Cell* **68**, 237–255 (1992).
- Rhyu, M. S., Jan, L. Y. & Jan, Y. N. *Cell* **76**, 477–491 (1994).
- Spana, E. P. & Doe, C. Q. *Development* **121**, 3187–3195 (1995).
- Kemphues, K. J., Priess, J. R., Morton, D. G. & Cheng, N. *Cell* **52**, 311–320 (1988).
- Kirby, C., Kusch, M. & Kemphues, K. *Dev Biol.* **142**, 203–215 (1990).
- Morton, D. G., Roos, J. M. & Kemphues, K. J. *Genetics* **130**, 771–790 (1992).
- Levitani, D. J., Boyd, L., Mello, C. C., Kemphues, K. J. & Stinchcomb, D. T. *Proc. natn. Acad. Sci. U.S.A.* **91**, 6108–6112 (1994).
- Cheng, N. N., Kirby, C. M. & Kemphues, K. J. *Genetics* **139**, 549–559 (1995).
- Guo, S. & Kemphues, K. J. *Cell* **81**, 611–620 (1995).
- Etemad-Moghadam, B., Guo, S. & Kemphues, K. J. *Cell* **83**, 743–752 (1995).
- Blancar, M. A. & Rutter, W. J. *Science* **256**, 1014–1018 (1992).
- Young, P. E., Richman, A. M., Ketchum, A. S. & Kiehart, D. P. *Genes Dev.* **7**, 29–41 (1993).
- Lin, R., Thompson, S. & Priess, J. *Cell* **83**, 599–609 (1995).
- Spudich, J. A. *Cell Regul.* **1**, 1–11 (1989).
- Kiehart, D. P. *Cell* **60**, 347–350 (1990).
- Hill, D. P. & Strome, S. *Dev Biol.* **125**, 75–84 (1988).
- Hill, D. P. & Strome, S. *Development* **108**, 159–172 (1990).

- Drubin, D. G. & Nelson, W. J. *Cell* **84**, 335–344 (1996).
- Jansen, R., Dowzer, C., Michaelis, C., Galova, M. & Nasmyth, K. *Cell* **84**, 687–697 (1996).
- Bobola, N., Jansen, R., Shin, T. H. & Nasmyth, K. *Cell* **84**, 699–709 (1996).
- Simons, M. *et al. Circulation Res.* **69**, 530–539 (1991).
- Shohet, R. V. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 7726–7730 (1989).
- Ketchum, A. S., Stewart, C. T., Stewart, M. & Kiehart, D. P. *Proc. natn. Acad. Sci. U.S.A.* **87**, 6316–6320 (1990).
- Wilson, R. *et al. Nature* **368**, 32–38 (1994).
- Okkema, P. G. & Fire, A. *Development* **120**, 2175–2186 (1994).
- Sulston, J., Schierenberg, E., White, J. & Thomson, N. *Dev Biol.* **100**, 67–119 (1983).

ACKNOWLEDGEMENTS. We thank M. Blancar for plasmids and communications about interaction cloning; P. Okkema for the *C. elegans* λ gt11 cDNA expression library; Y. Kohara for cDNA clones of the *nmy-1* gene; L. Boyd and B. Etemad-Moghadam for anti-PAR-2 and anti-PAR-3 antibodies; D. Kiehart and colleagues for discussion; A. Brestcher, T. Fox and M. Wolfner for comments on the manuscript. S. Guo thanks B. Lu for advice. This work was supported by a grant from the NIH.

CORRESPONDENCE and requests for materials should be addressed to K.J.K. (e-mail: kjk1@cornell.edu). The *nmy-2* gene sequence has been deposited in the Genbank database, accession number U49263.

Symmetry perception in an insect

Martin Giurfa, Birgit Eichmann & Randolph Menzel

Institut für Neurobiologie, Freie Universität Berlin,
Königin-Luise-Strasse 28/30, 14195 Berlin, Germany

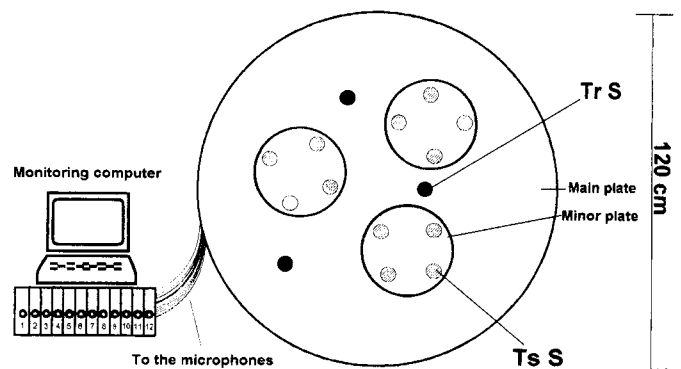
SYMMETRICAL visual patterns have a salient status in human perception, as evinced by their prevalent occurrence in art¹, and also in animal perception, where they may be an indicator of phenotypic and genotypic quality^{2–4}. Symmetry perception has been demonstrated in humans^{5–8}, birds^{9–11}, dolphins¹² and apes¹³. Here we show that bees trained to discriminate bilaterally symmetrical from non-symmetrical patterns learn the task and transfer it appropriately to novel stimuli, thus demonstrating a capacity to detect and generalize symmetry or asymmetry. We conclude that bees, and possibly flower-visiting insects in general, can acquire a generalized preference towards symmetrical or, alternatively, asymmetrical patterns depending on experience, and that symmetry detection is preformed or can be learned as a perceptual category by insects, because it can be extracted as an independent visual pattern feature. Bees show a predisposition for learning and generalizing symmetry because, if trained to it, they choose it more frequently, come closer to and hover longer in front of the novel symmetrical stimuli than the bees trained for asymmetry do for the novel asymmetrical stimuli. Thus, even organisms with comparatively small nervous systems can generalize about symmetry, and favour symmetrical over asymmetrical patterns.

Our aim was to examine whether bees can be trained to discriminate patterns purely on the basis of their symmetry, without reference to memorized images. The question of symmetry perception is relevant in an ecological and evolutionary context because bees forage on flowers that display remarkable levels of symmetry^{14–15}. However, whether pollinators, like the honeybee, can actually be trained to discriminate patterns purely on the basis of their symmetry is an open question. Here we investigate this question using bilaterally symmetrical and asymmetrical patterns. The patterns were presented in the vertical plane so that their appearance was independent of approach direction¹⁶. Such an arrangement is ecologically relevant because bilaterally symmetrical flowers mostly present themselves vertically, whereas radially symmetrical flowers tend to present themselves horizontally¹⁴.

Our experiment consisted of presenting the bees with a succession of different stimuli that remained constant only in their degree of symmetry. To test whether the insects were capable of extracting this feature, they were presented with novel stimuli that could be distinguished only on the basis of their symmetry. If the bees selectively choose the novel stimuli displaying the rewarded feature, it is possible to affirm that they can abstract the critical information and transfer it to new patterns. Such a design has been used successfully with pigeons¹⁰ and dolphins¹² trained to distinguish symmetrical and asymmetrical patterns, and with honeybees¹⁷ trained to distinguish pattern orientation.

Single, individually marked, free-flying honeybees were trained to forage on a vertically arranged, circular patch with multiple feeding sites¹⁸ (Fig. 1). The symmetrical group of bees was trained with a succession of eight diverse triads, each consisting of a rewarded symmetrical stimulus and two different non-rewarded asymmetrical stimuli presented simultaneously. The asymmetrical group was similarly trained, but with the asymmetrical stimulus

FIG. 1 A multiple choice arrangement to test the choice performance of a single bee using a set of microphones. The main, vertical, round plate (1.2 m diameter, with its centre 1.5 m above the ground) contained 3 training sites (Tr S: black) and 12 test sites (Ts S: hatched), all 7 cm in diameter. During training, only the 3 training sites were visible on the main plate, but not the 12 test sites, and vice versa during tests. The training sites were at different distances from the centre of the main plate to avoid learning of position cues; the test sites were arranged in three smaller subplates, 30 cm diameter (4 per subplate). Both the main and the smaller plates could be rotated independently. Position of the training stimulus was constantly exchanged and the main plate was always randomly rotated. Positions of the test stimuli were changed by rotating the main as well as the minor plates. Each test site was equipped with a pair of microphones for automatic detection of the approaching bee during tests; frequency and intensity thresholds were adjusted to equalize the sensitivity and to detect the bee's flight noise from a distance of 5 cm. The signals of the microphones were converted from an analogue to a digital signal and monitored by a computer with a time resolution of 11 ms. A signal from a given microphone lasting longer than 33 ms was recorded as a choice. For each test stimulus, the number of choices by the bee, the choice intensity, (inversely proportional to the distance from the stimulus) and the duration



of each choice were recorded. Stimuli were covered by Plexiglas disks so that bees could neither directly contact nor scent-mark the patterns used. These disks were always carefully washed.

rewarded and the two symmetrical stimuli unrewarded (Fig. 2a). Bees were tested with twelve novel stimuli (Fig. 2b) in unrewarded, multiple-choice, generalization tests that were interspersed with triad training.

Care was taken to ensure that pattern parameters other than symmetry were randomized during training, so that symmetry was the critical feature associated with reward (Fig. 3). To this aim,

parameters that bees are known to employ when discriminating patterns¹⁶ were quantified. Area, contour length and contour density¹⁹ (the ratio of area to contour length) were measured directly on the patterns, and the visual angle subtended by the main axis of the pattern²⁰ at the recording point was established. Two-dimensional fast Fourier transform amplitude spectra of the patterns were produced and total power²¹ (total energy change

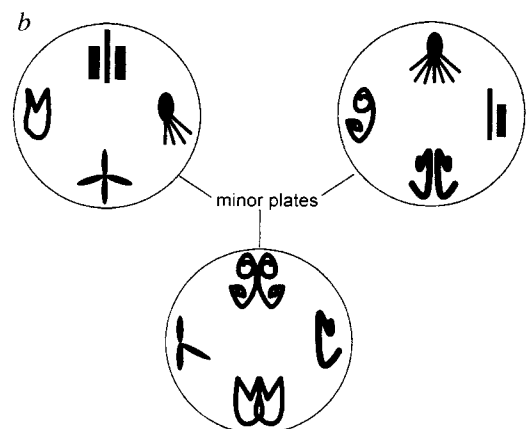
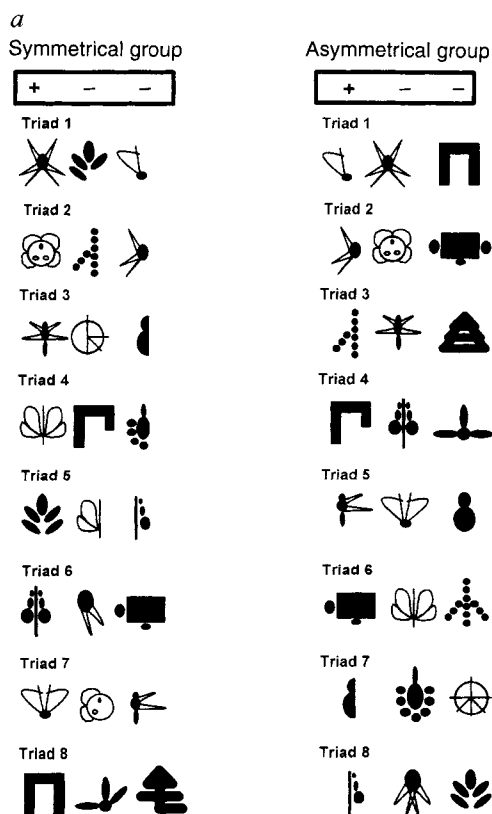
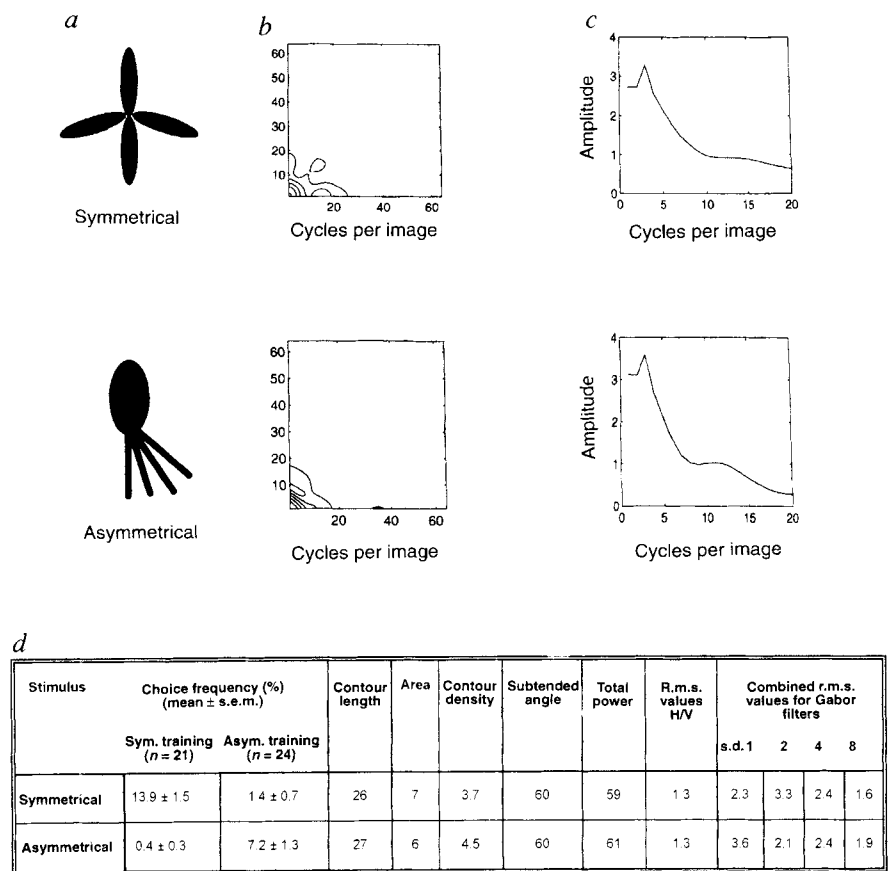


FIG. 2 Training and test stimuli were black and presented against a bee-white background. They were very diverse, except in their symmetrical or asymmetrical outline (see Fig. 3). a, Training triads for the symmetrical and the asymmetrical group of bees. +, rewarded stimulus; -, unrewarded stimuli. b, Test stimuli arranged on the 3 minor plates. On each plate, two symmetrical and 2 asymmetrical stimuli were presented.

METHODS. Single individually marked, free-flying honeybees were trained

with successive triads of stimuli. Two groups of 4–5 bees were established according to the training procedure. The symmetrical group was trained with a succession of 8 triads, each consisting of a rewarded symmetrical stimulus and two different unrewarded asymmetrical stimuli presented simultaneously. The asymmetrical group was similarly trained but with the asymmetrical stimulus rewarded and the two symmetrical stimuli unrewarded. Successive triads were composed of new patterns and did not contain any of the previously trained stimuli. Stimuli forming the training triads were different from those used in tests. Six respective control groups of 3–5 bees (symmetrical and asymmetrical) were run: in two of them, the succession of training triads was randomized with respect to the fixed one (see Fig. 4d); in other two, the area of the stimuli was varied by means of a computer program in such a way that all training and test patterns had exactly the same area (6 cm²), and the succession of training triads was again randomized; in the last two, the visual angle subtended by the patterns at the recording point was varied in such a way that all training and test patterns subtended exactly the same angle (65°). Ten to ninety learning trials per triad were carried out, until a total number of 270 was reached. Training with each triad was interspersed with multiple-choice, generalization tests (a total of 9 tests). During tests, 12 novel test stimuli that remained constant throughout the experiment were presented for 2 min on the test sites. Six were symmetrical and 6 asymmetrical, none was rewarded. For each test stimulus, the number of choices by the bee, the intensity and duration of each choice were recorded.

FIG. 3 An example of the analysis performed in the test patterns to check that symmetry was the critical cue allowing discrimination during the generalization tests. Two-dimensional fast Fourier transform amplitude spectra of the patterns were obtained and amplitude spectra were averaged across all orientations. Patterns were convoluted with broad band, highly oriented filters to measure power in the horizontal, vertical and diagonal (positive and negative) orientations. The filters had the form of a gaussian line set in negative circular gaussian which gives zero d.c. Through this analysis, root mean square (r.m.s.) values, a conventional measure of power in signals, were obtained. The vertical filter measures energy in the horizontal axis and gives the horizontal r.m.s. power; the horizontal filter measures energy in the vertical axis and gives the vertical r.m.s. power. Patterns were also convoluted with horizontally and vertically oriented, symmetrical (cos) and asymmetrical (sin) Gabor functions at four spatial scales²³. The inclusion of these scales is relevant because changes in resolution may affect pattern recognition. The standard deviation (s.d.) of the Gabor gaussian envelope is given in pixels (1, 2, 4 and 8) and the spatial period with which it is multiplied is given by s.d./0.2 (5, 10, 20 and 40 pixels, respectively). Convolutions with symmetrical and asymmetrical Gabor functions were combined to compare horizontal and vertical r.m.s. signal power at the four spatial scales. *a*, A symmetrical and an asymmetrical test pattern. *b*, Two-dimensional fast Fourier transform amplitude spectra of the test patterns; 64 cycles per image equates to 0.5 cycles per pixel. *c*, Amplitude spectra averaged across all orientations. *d*, Choice frequencies of the bees after symmetrical and asymmetrical training, after the generalization performance has been established (test 7 onwards) and the parameters that were considered for pattern analysis: contour length (cm); area (cm²); contour density¹⁹ (cm⁻¹); angle subtended by the main axis of the pattern²⁰ at the recording point (°); total power²¹; r.m.s. values for the ratio of horizontal to vertical (H/V) power; and combined symmetrical and asymmetrical r.m.s. values for the vertical/horizontal ratio of Gabor filter orientations at four spatial scales (s.d. 1, 2, 4



across the pattern) and orientational power were calculated. As bees are especially sensitive to orientation cues¹⁷, orientational power was calculated by convoluting the patterns with broad-band, highly oriented vertical, horizontal and diagonal filters²², and with symmetrical and asymmetrical, vertically and horizontally oriented Gabor functions^{23,24} at four different spatial scales. Such analysis was focused on the test patterns of Fig. 2b because these are the stimuli the bees should discriminate as symmetrical and asymmetrical. These analyses did not reveal critical differences between the two classes of patterns other than the degree of symmetry, which would be the reliable cue to distinguish between stimulus categories. When significant differences other than the degree of symmetry arose, the bees' responses were examined to test whether such differences accounted for their stimulus choice. In no cases were the bees' performances correlated with differences in other features.

Bees trained either to the symmetrical or asymmetrical patterns were able to choose consistently the novel symmetrical or asymmetrical patterns, respectively, in generalization tests (Fig. 4). Three measures of performance (choice frequency, choice intensity and choice duration) gave concordant results. A significant deviation from random choices occurred from the seventh test onwards (Fig. 4a–c), from which point bees preferred to approach the trained feature, came closer to it and inspected it for longer. In all three cases, there were no significant differences between the symmetrical and the asymmetrical group in the first test. Thus,

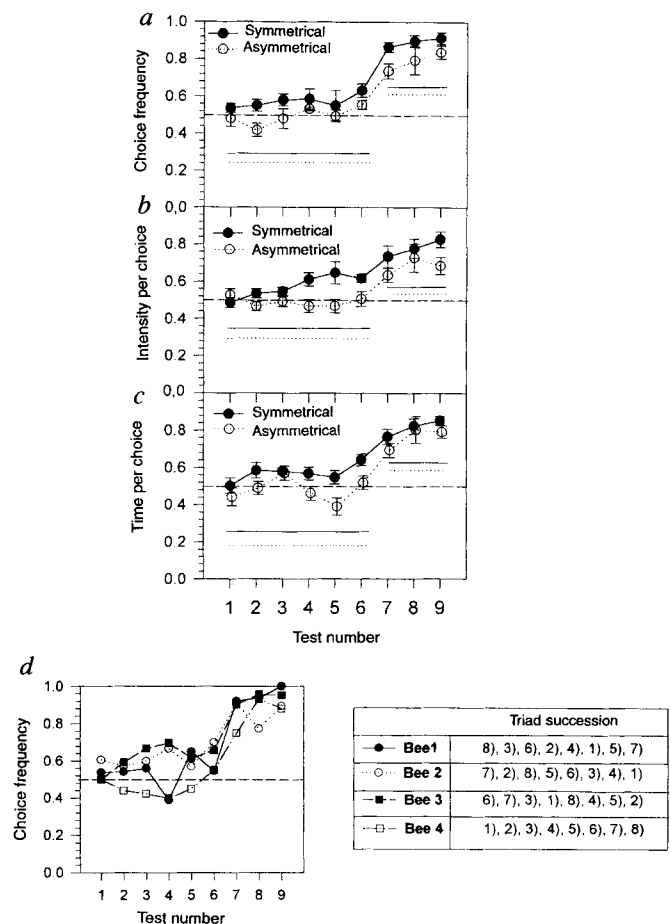
and 8)²³. As shown by this example, there were no marked differences between these patterns, even considering the four different scales employed. When significant differences other than degree of symmetry arose, the bees' responses were examined to test whether such differences accounted for their stimulus choice. In no case could the discrimination of symmetrical and asymmetrical patterns by bees be correlated with differences in a feature other than symmetry.

bees did not show 'spontaneous' preferences either for symmetry or asymmetry at the beginning of the test session, and the later preferences were experience dependent. However, for all three parameters, the responses of the symmetrical and the asymmetrical group differed significantly, with the 'symmetrical' bees performing better than the 'asymmetrical' ones. This result demonstrates that bees have a predisposition for learning and extracting symmetry as a feature.

We conclude that honeybees can abstract the cue 'symmetry' and thus generalize it as an image feature. As the abstraction of symmetry made by bees was experience dependent, it was not possible to decide whether it reflected the existence of an innate or a learned symmetry category. 'Symmetrical' bees may have performed better than 'asymmetrical' ones because of an innate predisposition to learn better and faster about stimuli that are biologically relevant^{25–26}, or because they transferred past experience of predominantly symmetrical flowers in the field. On the other hand, the predisposition for symmetrical patterns is also consistent with the observation that bees spontaneously fly towards symmetrical patterns^{27,28} and with the suggestion that symmetry preferences arise as a by-product of the need to recognize objects irrespective of their position and orientation in the visual field²⁹.

In the training set that we used, bees did not merely learn the symmetry feature, but they generalized it to novel stimuli. This finding clearly differentiates our results from previous ones: although it has been shown that bees may prefer symmetrical

FIG. 4 Choice frequency, intensity and time for both the symmetrical and the asymmetrical group of bees during the successive tests. For each test, these parameters were computed for each stimulus and normalized to 1. The values obtained were added for the 6 symmetrical and the 6 asymmetrical stimuli, respectively. The hatched line at 0.5 indicates no preference for either group of stimuli. Data were analysed by repeated measurements ANOVA, with 'symmetry' as a two-level fixed effect (symmetrical or asymmetrical training). Horizontal lines connect points that do not differ at the 5% level (Newmann-Keuls test); solid line: symmetrical group; dotted line: asymmetrical group. For both groups of bees, there were no significant differences in all three parameters according to whether bees were trained with the fixed or with the randomized succession of triads (repeated measurements ANOVA with 'triad succession' as a two-level fixed effect (fixed or random). Thus, results of both procedures were pooled. Symmetrical group: $n = 1,623$ choices (7 bees); asymmetrical group: $n = 1,438$ choices (8 bees). Controls where the areas or the subtended angles of both the training and the test stimuli were the same, showed that these parameters did not influence performance (not shown). a, The mean relative choice frequency ($\pm$ s.e.m.) for the trained feature. Bees extracted the symmetry or asymmetry feature and transferred it to the novel stimuli ($F = 34.5$; d.f., 8, 96; $P < 0.0001$) from test 7 onwards. Curves of the symmetrical and the asymmetrical group significantly differ ($F = 7.4$; d.f., 1, 96; $P < 0.02$), with the bees trained for symmetry choosing more frequently the novel symmetrical stimuli than the bees trained for asymmetry did with the novel asymmetric stimuli. The interaction was not significant ($F = 0.3$; d.f., 8, 96; n.s.). b, The mean relative intensity per choice ($\pm$ s.e.m.) for the trained feature. Bees extracted the symmetry or asymmetry feature by coming closer to the trained feature from test 7 onwards ($F = 14.7$; d.f., 8, 96; $P < 0.0001$). The curves significantly differ ($F = 10$; d.f., 1, 96; $P < 0.01$) with the bees hovering closer to the novel symmetrical stimuli when trained for symmetry, than to the asymmetrical ones when trained for asymmetry. The interaction was not significant ($F = 1.5$; d.f., 8, 96; n.s.). c, The mean relative time per choice ($\pm$ s.e.m.) for the trained feature. Bees extracted the symmetry or asymmetry feature by hovering longer at the trained feature from test 7 onwards ($F = 26.1$; d.f., 8, 96; $P < 0.0001$). Moreover, bees hovered significantly longer in front of the symmetrical stimuli when trained for this feature, than in front of the asymmetrical ones when trained for asymmetry ($F = 9.4$; d.f., 1, 96; $P = 0.01$). The interaction was not significant ($F = 0.8$; d.f., 8, 96; n.s.). d, Choice frequencies of four different bees rewarded for symmetry. Each bee received a different succession of training triads (right panel: triad numbers refer to those of Fig. 2b). Independently of



the succession of triads, the bees were capable of extracting the symmetry feature and choosing the new symmetrical patterns, from test 7 onwards.

patterns^{27,28}, this constitutes the first evidence that they may abstract symmetry as a feature, as a result of their experience. Our results also pose the question of whether bees were categorizing stimuli on the basis of a symmetry concept, raising the possibility that 'cognitive' capabilities may occur in organisms with rather small nervous systems. There may be a simple mechanistic explanation, that central neuronal elements comparing the visual input from the right and the left eye pixel-by-pixel are all that are necessary to perceive the symmetry of shapes. Another attractive explanation is that axes of bilateral symmetry can be found without pattern matching by a local mechanism that identifies edges and lines by classifying relative phases in spatial harmonics²⁴. For such a mechanism, an axis of symmetry appears as a line with no contrast. By means of their line and edge detectors³⁰, insects may perceive no more than the axis itself and thus learn to discriminate patterns on the basis of the axis detectability.

The perception of symmetry is important for pollinators because symmetry of a flower may signal its quality²⁸, and the reproductive success of plants is affected by the behaviour of pollinators. As pollinators in general may discriminate perceptually between symmetry and asymmetry, they should also be capable of performing selective pollination with respect to floral symmetry²⁸. This suggests that plants may have exploited the cognitive capabilities of the pollinators during the evolution of flowers. □

Received 6 February; accepted 3 June 1996.

1. Caglioti, G. *Symmetriebrechung und Wahrnehmung* (Vieweg, Braunschweig, 1990).
2. Möller, A. P. *Nature* **357**, 238–240 (1992).

3. Möller, A. P. *Behav. Ecol. Sociobiol.* **32**, 371–376 (1993).
4. Swaddle, J. P. & Cuthill, I. *Nature* **367**, 165–166 (1994).
5. Corballis, M. C. & Roldán, C. E. *J. exp. Psychol.* **1**, 221–230 (1975).
6. Pashler, H. *J. exp. Psychol.* **16**, 150–163 (1990).
7. Barlow, H. B. & Reeves, B. C. *Vision Res.* **19**, 783–793 (1979).
8. Bornstein, M. H., Ferdinandsen, K. & Gross, C. G. *Dev. Psychol.* **17**, 82–86 (1981).
9. Delius, J. D. & Habers, G. *Behav. Biol.* **22**, 336–342 (1978).
10. Delius, J. D. & Nowak, B. *Psychol. Res.* **44**, 199–212 (1982).
11. Menne, M. & Curio, E. *Z. Tierpsychol.* **47**, 299–322 (1978).
12. von Fersen, L., Manos, C., Goldowsky, B. & Roitblat, H. in *Marine Mammal Sensory Systems* (ed. Thomas, J. et al.), 753–762 (Plenum, New York, 1992).
13. Rensch, B. *Z. Tierpsychol.* **14**, 71–99 (1957).
14. Menzel, R. & Shmida, A. *Biol. Rev.* **68**, 81–120 (1993).
15. Möller, A. P. & Eriksson, M. *J. evol. Biol.* **7**, 97–113 (1994).
16. Wehrer, R. in *Handbook of Sensory Physiology* (ed. Autrum, H. J.), Vol. VII/6C, 287–616 (Springer, Berlin, 1981).
17. van Hateren, J. H., Srinivasan, M. & Wait, P. B. *J. comp. Physiol.* **A167**, 649–654 (1990).
18. Giurfa, M., Backhaus, W. & Menzel, R. *Naturwissenschaften* **82**, 198–201 (1995).
19. Hertz, M. *Z. vergl. Physiol.* **8**, 693–748 (1929).
20. Horridge, G. A., Zhang, S. W. & Lehrer, M. *Phil. Trans. R. Soc. Lond.* **B337**, 49–57 (1992).
21. Srinivasan, M. *J. Insect Physiol.* **40**, 183–194 (1994).
22. Srinivasan, M., Zhang, S. & Witney, K. *Phil. Trans. R. Soc. Lond.* **B343**, 199–210 (1994).
23. Marčelja, S. *J. opt. Soc. Am.* **70**, 1297–1300 (1980).
24. Osorio, D. *Proc. R. Soc. B263*, 105–110 (1996).
25. Menzel, R. *Z. vergl. Physiol.* **56**, 22–62 (1967).
26. Giurfa, M., Núñez, J. A., Chittka, L. & Menzel, R. *J. comp. Physiol.* **A177**, 247–259 (1995).
27. Lehrer, M., Horridge, G. A., Zhang, S. W. & Gadagkar, R. *Phil. Trans. R. Soc. Lond.* **B347**, 123–137 (1995).
28. Möller, A. P. *Proc. natn. Acad. Sci. U.S.A.* **92**, 2288–2292 (1995).
29. Enquist, M. & Arak, A. *Nature* **372**, 169–172 (1994).
30. O'Carroll, D. *Nature* **362**, 541–543 (1993).

ACKNOWLEDGEMENTS. We thank D. Osorio for invaluable advice on analyses of the stimulus patterns, fruitful discussions and corrections on early versions of the manuscript. We also thank J. Delius for his corrections of an early version of the manuscript, R. Brandt and M. Vorobyev for suggestions and stimulating discussions, R. Dens for help with some control experiments, T. Faber and H. Jander for building the test apparatus and G. de Brito Sanchez, M. Hammer and J. Klein for encouragement. M. Giurfa was supported by the Alexander von Humboldt-Stiftung and by the International Foundation of Science (Stockholm, Sweden).

CORRESPONDENCE and requests for materials should be addressed to M.G. (e-mail: giurfa@neuro.biologie.fu-berlin.de).

Affinity maturation without germinal centres in lymphotoxin- α -deficient mice

Mitsuru Matsumoto*, Stanley F. Lo†, Cynthia J. L. Carruthers‡, Jingjuan Min‡, Sanjeev Mariathasan*, Guangming Huang*, David R. Plas‡, Steven M. Martin‡, Raif S. Geha§, Moon H. Nahm*† & David D. Chaplin*‡

* Center for Immunology and Department of Internal Medicine, † Department of Pathology, and ‡ Howard Hughes Medical Institute, Washington University School of Medicine, St Louis, Missouri 63110, USA § Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02138, USA

AFFINITY maturation by somatic hypermutation is thought to occur within germinal centres¹⁻⁴. Mice deficient in lymphotoxin- α ($LT\alpha^{-/-}$ mice) have no lymph nodes or Peyer's patches^{5,6}, and fail to form germinal centres in the spleen⁷. We tested whether germinal centres are essential for maturation of antibody responses to T-cell-dependent antigens. $LT\alpha^{-/-}$ mice immunized with low doses of (4-hydroxy-3-nitrophenyl)acetyl-ovalbumin (NP-OVA) showed dramatically impaired production of high-affinity anti-NP IgG1. However, $LT\alpha^{-/-}$ mice immunized with

high doses of NP-OVA, even though they failed to produce germinal centres, manifested a high-affinity anti-NP IgG1 response similar to wild-type mice. Furthermore, when $LT\alpha^{-/-}$ mice were multiply immunized with high doses of NP-OVA, the predominantly expressed anti-NP V_H gene segment *V_H186.2* showed somatic mutations typical of affinity maturation⁸. Thus, B-cell memory and affinity maturation are not absolutely dependent on the presence of germinal centres.

The failure of $LT\alpha^{-/-}$ mice to develop germinal centres persists even after multiple immunizations with high doses (200 μ g per injection) of the T-cell-dependent antigen NP-OVA adsorbed to alum (Fig. 1a, b). Flow cytometry using peanut agglutinin (PNA)⁴ can quantify germinal-centre B cells after immunization. Wild-type mice showed clear induction of PNA^{high} B cells both after single (data not shown) and multiple immunizations with NP-OVA adsorbed to alum (Fig. 1c); in contrast, no PNA^{high} B cells were detected in the $LT\alpha^{-/-}$ mice. Similar results were obtained after two immunizations with another T-cell-dependent antigen keyhole-limpet haemocyanin (KLH), injected without adjuvant (data not shown). Thus, $LT\alpha^{-/-}$ mice fail to form germinal centres even after repeated immunizations with T-cell-dependent antigens, with or without adjuvant.

Both primary and secondary lymphoid follicles characteristically contain follicular dendritic cells (FDCs)⁹. FDCs trap and can retain antigen-antibody complexes for long periods¹⁰, apparently by means of receptors (CR1, CR2 and CR3) for the third complement component⁹. FDCs in spleen follicles can be visualized immunohistochemically by using 8C12, a monoclonal

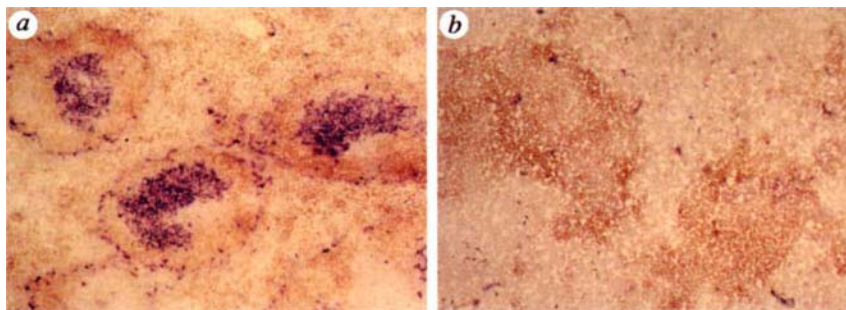


FIG. 1 Absence of germinal centres in the spleen of multiply immunized $LT\alpha^{-/-}$ mice. **a**, Wild-type (WT) and **b**, $LT\alpha^{-/-}$ mice were multiply immunized with NP-OVA, and spleen sections stained with PNA (blue) and anti-IgD (brown). Germinal centres are prominent in WT mice, but typical germinal-centre development is absent in $LT\alpha^{-/-}$ mice. Original magnification, $\times 100$. **c**, PNA^{high} B cells were not induced from $LT\alpha^{-/-}$ mice after multiple immunizations with NP-OVA adsorbed to alum.

METHODS. Mice were immunized i.p. with 200 μ g NP-OVA adsorbed to alum, and boosted twice using the same dose. Spleens were collected 13 days after the final boost, and frozen sections stained with PNA (Vector) and polyclonal rat anti-IgD serum (Southern Biotechnology) as described⁷. For flow cytometry (c), mice were immunized with NP-OVA as above, and spleen cell suspensions were stained with PNA-fluorescein isothiocyanate (Vector) and B220-phycoerythrin (PharMingen), as described⁴. Cells were analysed using a FACScan (Becton Dickinson) with CELLQuest software. Percentages of PNA^{high} B220⁺ cells relative to total B220⁺ cells are shown.

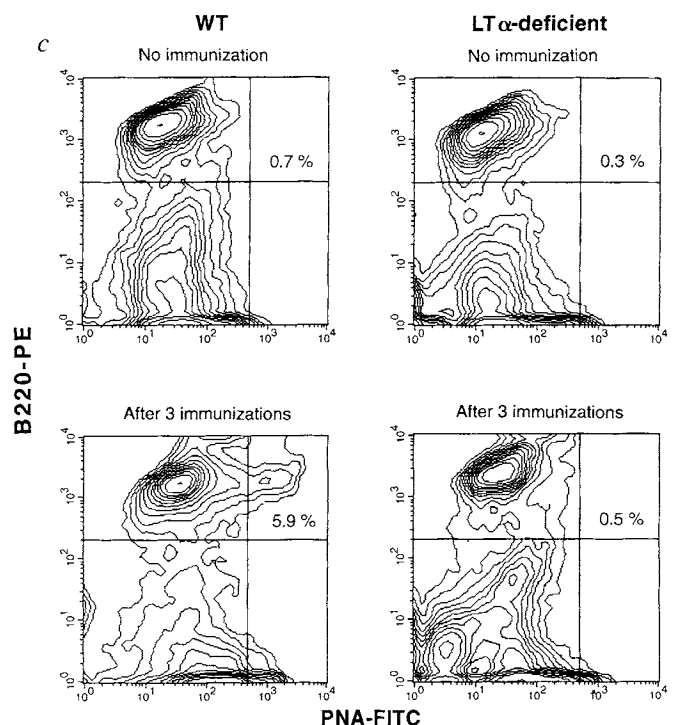
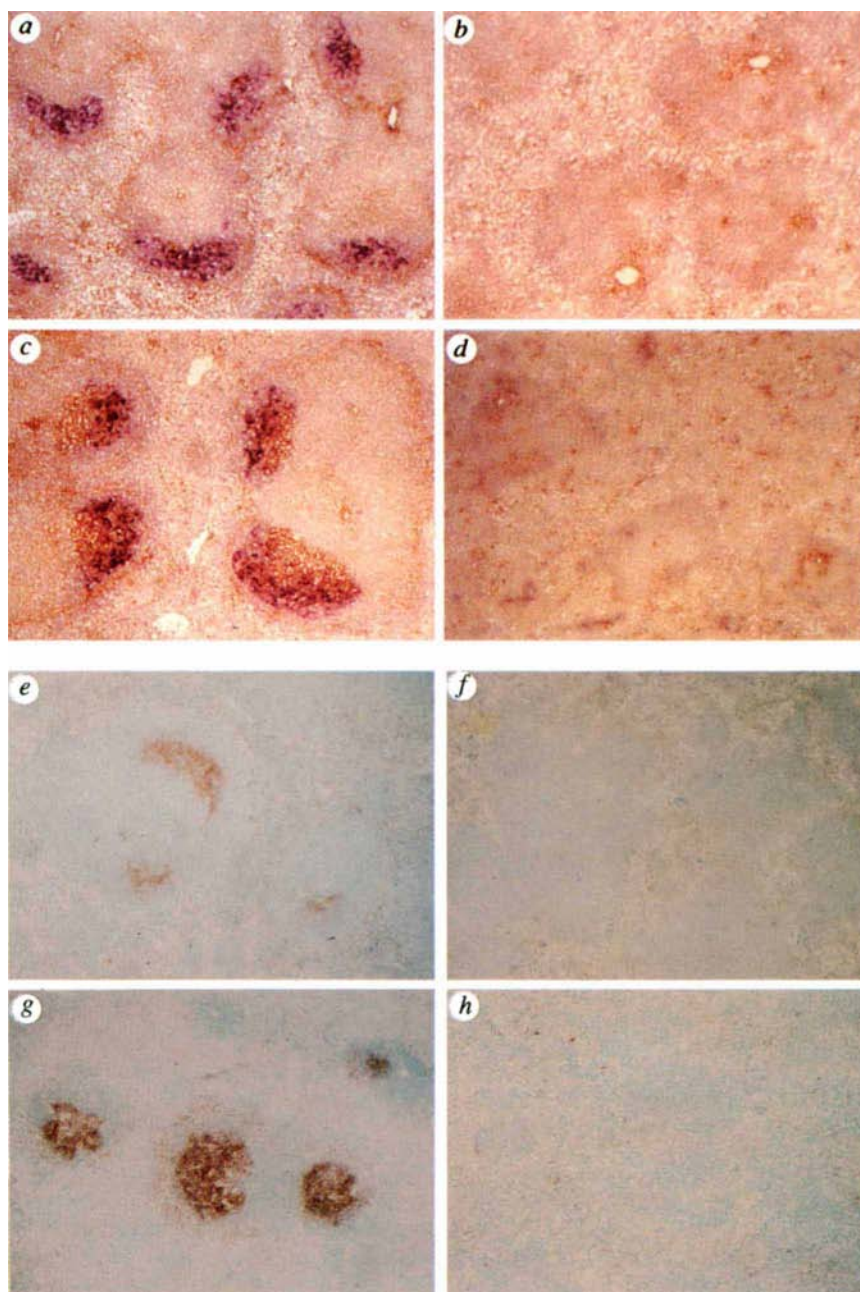


FIG. 2 Organization of FDCs is disturbed in $LT\alpha^{-/-}$ mice. Clustering of FDCs was detected with anti-CR1 monoclonal antibody (mAb) (blue staining) from unimmunized WT mouse spleen (a), and occupied the light zone of germinal centres (brown staining with PNA) after immunization with NP-OVA (c). $LT\alpha^{-/-}$ spleen showed no staining with anti-CR1 mAb or PNA, before (b) or after (d) immunization. PAP complexes either injected into the WT mice (e) or incubated *in vitro* with sections of WT spleen (g) were trapped by the spleen follicles. The spleen follicles of $LT\alpha^{-/-}$ mice failed to show trapping of PAP complexes either injected *in vivo* (f) or incubated *in vitro* (h). Original magnification, $\times 100$.

METHODS. Immunization of mice with NP-OVA and immunohistochemistry using PNA and anti-CR1 mAb (8C12)¹¹ were performed as described in Fig. 1. For e, f, rabbit PAP complex (200 μ l) (DAKO A/S; code Z 113, lot 082) was injected intravenously into mice which had been immunized 10 days previously with sheep red blood cells (100 μ l of a 10% suspension in PBS, pH 7.4), and spleens were collected 24 h later. Frozen sections of spleen were prepared and further incubated with horseradish peroxidase (HRP) conjugated goat anti-rabbit serum (Southern Biotechnology). After washing in PBS, colour development for bound HRP was performed using diaminobenzidine. For g, h, immune complex trapping *in vitro* was performed as described²⁶. Frozen sections of spleen were incubated with 1:10 diluted mouse PAP (DAKO A/S; code B650, lot 035A) in the presence of 1:5 diluted fresh mouse serum as a source of complement. After washing in PBS, colour was developed as described above.



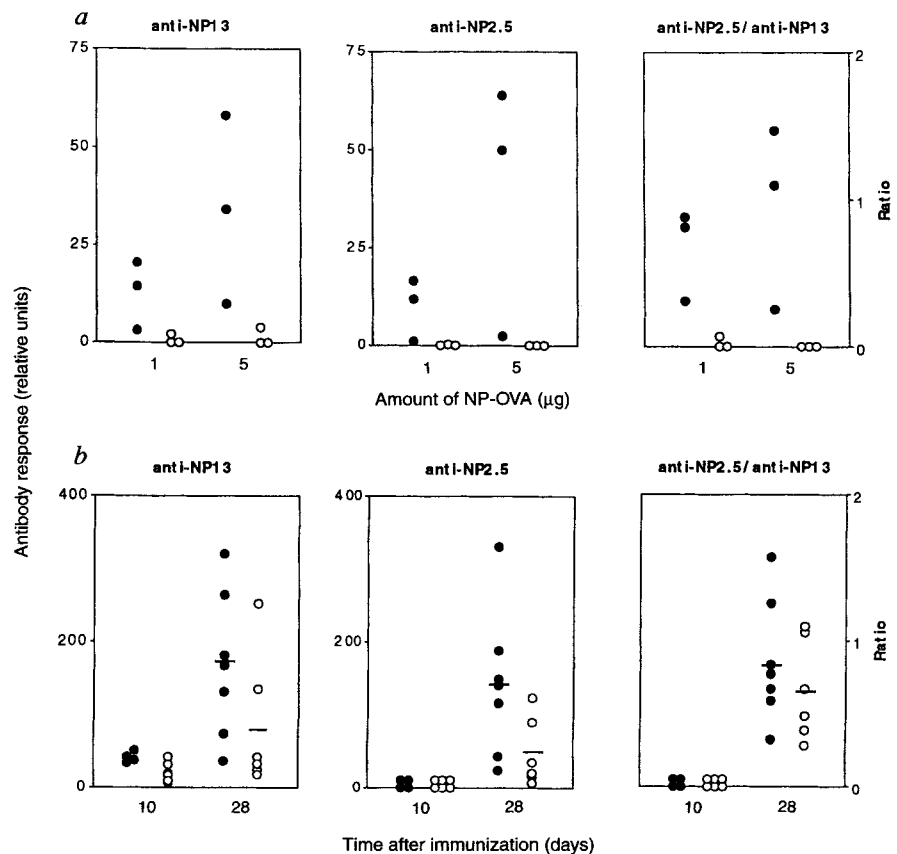
antibody specific for murine CR1 (ref. 11). In spleen of naive wild-type mice, FDC reticula were prominently stained (Fig. 2a); after immunization, the FDC constituted the germinal-centre light zone (Fig. 2c). In contrast, naive (Fig. 2b) and immunized (Fig. 2d) $LT\alpha^{-/-}$ mice showed no clusters of 8C12-staining cells. Failure of normal FDC organization was further demonstrated by analysis of immune complex trapping. Spleen follicles in wild-type mice trapped preformed horseradish peroxidase–anti-peroxidase (PAP) complexes either *in vivo* or *in vitro*, at sites corresponding to the FDC reticula (Fig. 2e, g). Immune complex trapping was not detected in $LT\alpha^{-/-}$ mice (Fig. 2f, h). Thus in $LT\alpha^{-/-}$ mice, organized FDC structure, a major morphological characteristic of germinal centres, is absent.

We tested whether germinal centres are essential for the maturation of antibody responses to T-cell-dependent antigens. $LT\alpha^{-/-}$ and wild-type mice that had not been immunized showed similar levels of total serum IgM and IgG (data not shown). Anti-NP antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) following intraperitoneal immunization of

mice with NP-OVA. When the ELISA plate was coated with densely NP-haptenated bovine serum albumin (NP₁₃-BSA), both high- and low-affinity anti-NP antibodies were bound. When the plate was coated with sparsely haptenated BSA (NP₂₅-BSA), only high-affinity anti-NP antibodies were bound. Therefore, the appearance of NP₂₅-binding antibodies and an increased ratio of NP₂₅-binding/NP₁₃-binding antibody demonstrate affinity maturation^{12,13}. When immunized twice with either 1 or 5 μ g of NP-OVA, all wild-type mice produced NP-specific IgG1 antibody and showed affinity maturation (Fig. 3a); by day 28, the average ratio of NP₂₅/NP₁₃-binding antibodies was 0.8. In contrast, only two of six $LT\alpha^{-/-}$ mice produced detectable anti-NP IgG1 by day 28, with no evidence of high-affinity antibody. The $LT\alpha^{-/-}$ mice showed anti-NP IgM responses equal to or greater than those of wild-type mice (data not shown). Thus, the impaired IgG1 response in $LT\alpha^{-/-}$ mice immunized with low doses of antigen did not result from a defect in antigen delivery.

High doses of NP-OVA (200 μ g) overcame this defective humoral response. After high-dose immunization, $LT\alpha^{-/-}$ mice

FIG. 3 Antigen-specific antibody responses in $LT\alpha^{-/-}$ mice vary according to the dosage of immunogen. **a**, Antibody response (amount of NP-specific serum IgG1) at day 28 in $LT\alpha^{-/-}$ mice following immunization with a low dose of NP-OVA. **b**, Antibody response in $LT\alpha^{-/-}$ mice after immunization with a high dose of NP-OVA. WT mice, filled circles; $LT\alpha^{-/-}$ mice, open circles. **METHODS.** Both WT and $LT\alpha^{-/-}$ mice were immunized i.p. with either: **a**, low doses (1 or 5 μ g per injection), or **b**, high doses (200 μ g per injection) of NP-OVA adsorbed to alum. Mice were boosted 21 days later using the same doses of antigen. Sera were collected 10 and 28 days after the initial immunization. NP-specific IgG1 antibody of low and high affinity was detected by ELISA with NP₁₃-BSA and NP_{2.5}-BSA coated plates, respectively^{12,13}. Antibodies bound to the plates were detected using a goat anti-mouse IgG1 antibody conjugated with HRP (Southern Biotechnology). The quantities of NP-specific serum IgG1 in each experiment were expressed in relative units compared to a standard hyperimmune mouse serum²⁹, we defined 100 units as the quantity of anti-NP antibody in a standard hyperimmune serum. When both anti-NP_{2.5} and anti-NP₁₃ antibodies were undetectable, the ratio of anti-NP_{2.5}/anti-NP₁₃ was defined as 0.



produced similar amounts of NP-specific IgG1 compared to wild-type mice, demonstrating that isotype switching can occur in the absence of germinal centres (Fig. 3b). Furthermore, the ratio of IgG1 antibody bound to NP_{2.5}-BSA and NP₁₃-BSA suggested similar acquisition of high-affinity antibody in wild-type and $LT\alpha^{-/-}$ mice. Thus affinity maturation is not absolutely dependent on germinal centres.

The locations of anti-NP IgG1-producing cells in high-dose NP-OVA-immunized wild-type and $LT\alpha^{-/-}$ mice were investigated using the ELISPOT assay¹³. Seven days after a single immunization, $LT\alpha^{-/-}$ mice showed no detectable anti-NP IgG1-producing cells in either peripheral blood or resident peritoneal cells (<1 anti-NP-producing cell per 10^6 leukocytes). Anti-NP IgG1-producing cells were detected at 10 per 10^5 cells in spleen. Wild-type mice showed no anti-NP IgG1-producing cells in peripheral blood or peritoneal cells, and 8 anti-NP IgG1-producing cells per 10^5 cells in spleen. These data suggest that the maturation of the anti-NP B-cell response occurs primarily in the spleen in both wild-type and $LT\alpha^{-/-}$ mice.

During the primary response to NP, the combination of λ 1 light chain and the heavy-chain variable region gene *VH186.2* predominates^{2,8,14-16}. This VH gene also predominates in secondary responses, but in somatically mutated forms^{8,15,17-19}. Mutation of Trp to Leu at codon 33 in the first complementarity determining region (CDR1) of the *VH186.2* gene is highly correlated with acquisition of higher affinity for NP^{8,20}. We examined the sequences of *VH186.2* IgG1 transcripts expressed in spleen after immunization with NP-OVA and found that immunized and boosted wild-type mice showed somatic mutations scattered throughout the *VH186.2* segment (Fig. 4a). Codon 33 in the CDR1 region showed mutation from Trp to Leu in 14 of 18 independent sequences, indicating affinity maturation by somatic mutation. Similarly immunized and boosted $LT\alpha^{-/-}$ mice also showed somatic mutation of the variable regions in RNA transcribed from this gene (Fig. 4b). The total number of somatically mutated nucleotides was only 30% of that in wild-type mice (Fig.

4c). Nevertheless, 9 of 18 independent sequences showed the codon 33 Trp to Leu mutation (Fig. 4b). Thus, although somatic mutation was reduced in the $LT\alpha^{-/-}$ mice, selection of cells expressing high-affinity variants occurred effectively. In addition, the mutations observed showed a similar pattern of strand bias, preference for transitions rather than transversions, and favouring of mutations at AGC/T sequences, as previously observed^{21,22}, suggesting that the mutational mechanism in the $LT\alpha^{-/-}$ mice has the same properties as that in wild-type mice.

The pattern of replacement and silent mutations in the expressed *VH186.2* sequences provides additional evidence of selection following mutation (Fig. 4c). If somatic mutations accumulated randomly, then the expected ratio of replacement to silent mutations in the CDR and the framework regions would be 4.9 and 2.6, respectively^{13,18}. In both $LT\alpha^{-/-}$ and wild-type mice, the observed replacement/silent value in CDR1 and CDR2 is higher than that expected from random mutagenesis, whereas the value observed in the framework regions approximates the expected value. These results suggest that, as in wild-type mice, somatic mutation in $LT\alpha^{-/-}$ mice is an antigen-driven process.

The absence of germinal centres in $LT\alpha^{-/-}$ mice is associated with failure to organize clusters of FDCs. Differentiation and/or organization of FDCs is thought to depend on interactions with both B and T cells²³⁻²⁶. Thus, disturbed organization of FDCs in $LT\alpha^{-/-}$ mice might not be the primary cellular defect that determines absence of germinal centres. However, the type I tumour-necrosis-factor receptor (TNFR-I), which binds the soluble $LT\alpha$ homotrimer, is expressed highly on FDCs in the spleen²⁷, and mice deficient for TNFR-I also fail to form both germinal centres⁷ and organized FDC clusters (data not shown). $LT\alpha$ -deficiency may result in failure to deliver a TNFR-I-mediated signal that is required for recruitment of FDCs, which in turn, may lead to failure to form germinal centres.

It is striking that high doses of antigen can activate the affinity maturation process even in the absence of germinal centres. Both somatic mutations of immunoglobulin genes and selection of

clones producing high-affinity antibodies were detected in $LT\alpha^{-/-}$ mice. Thus, this germinal-centre-independent mechanism possesses the essential characteristics of the germinal-centre-dependent affinity maturation process. It has been suggested that the selection of B lymphocytes expressing high-affinity mutants of their immunoglobulin chains occurs normally within the germinal centre by competition for antigen displayed on the surfaces of FDCs, and that B cells that are not selected die by apoptosis^{1,9,28}. Selection of high-affinity antibody-producing cells in $LT\alpha^{-/-}$ mice in the absence of either germinal centres or organized FDC clusters appears inconsistent with this model. However, although $LT\alpha^{-/-}$ mice contain no recognizable FDC clusters, there may be FDCs dispersed throughout the splenic follicles in sufficient numbers to support the selection process. Alternatively, there may be other cell populations that can substitute for FDCs. □

Received 13 March; accepted 3 June 1996.

1. MacLennan, I. C. M. *Rev. Immun.* **12**, 117–139 (1994).
2. Jacob, J., Kelsoe, G., Rajewsky, K. & Weiss, U. *Nature* **354**, 389–392 (1991).
3. Küppers, R., Zhao, M., Hansmann, M.-L. & Rajewsky, K. *EMBO J.* **12**, 4955–4967 (1993).
4. Berek, C., Berger, A. & Apel, M. *Cell* **67**, 1121–1129 (1991).
5. De Togni, P. *et al. Science* **264**, 703–707 (1994).
6. Banks, T. A. *et al. J. Immun.* **155**, 1685–1693 (1995).
7. Matsumoto, M. *et al. Science* **271**, 1289–1291 (1996).

8. Rajewsky, K., Forster, I. & Cumano, A. *Science* **238**, 1088–1094 (1987).
9. Schriever, F. & Nadler, L. M. *Adv. Immun.* **51**, 243–284 (1992).
10. Nossal, G. J., Abbot, A. & Mitchell, J. J. *exp. Med.* **127**, 263–276 (1968).
11. Kinoshita, T. *et al. J. Immun.* **140**, 3066–3072 (1988).
12. Roes, J. & Rajewsky, K. *J. exp. Med.* **177**, 45–55 (1993).
13. Smith, K. G. C., Weiss, U., Rajewsky, K., Nossal, G. J. V. & Tarlinton, D. M. *Immunity* **1**, 803–813 (1994).
14. Jack, R. S., Imanishi-Kan, T. & Rajewsky, K. *Eur. J. Immun.* **7**, 559–565 (1977).
15. Cumano, A. & Rajewsky, K. *EMBO J.* **5**, 2459–2468 (1986).
16. Blier, P. R. & Bothwell, A. L. M. *J. Immun.* **139**, 3996–4006 (1987).
17. Weiss, U. & Rajewsky, K. *J. exp. Med.* **172**, 1681–1689 (1990).
18. Weiss, U., Zobebein, R. & Rajewsky, K. *Eur. J. Immun.* **22**, 511–517 (1992).
19. McHeyzer-Williams, M. G., Nossal, G. J. V. & Lalor, P. A. *Nature* **350**, 502–505 (1991).
20. Allen, D. *et al. Immun. Rev.* **96**, 5–22 (1987).
21. Neuberger, M. S. & Milstein, C. *Curr. Opin. Immun.* **7**, 248–254 (1995).
22. Wagner, S. D., Milstein, C. & Neuberger, M. S. *Nature* **376**, 732 (1995).
23. Cerny, A., Zinkernagel, R. M. & Groscurth, P. *Cell Tiss. Res.* **254**, 449–454 (1988).
24. Clark, E. A., Grabstein, K. H. & Shu, G. L. *J. Immun.* **148**, 3327–3335 (1992).
25. Kapasi, Z. F., Burton, G. F., Shultz, L. D., Tew, J. G. & Szakal, A. K. *J. Immun.* **150**, 2648–2658 (1993).
26. Yoshida, K. *et al. Eur. J. Immun.* **24**, 464–468 (1994).
27. Ryffel, B. & Mihatsch, M. J. *Int. Rev. exp. Path.* **34B**, 149–156 (1993).
28. Liu, Y.-J. *et al. Nature* **342**, 929–931 (1989).
29. Hebel, T., Ahearn, J. M. & Fearon, D. T. *Science* **254**, 102–105 (1991).

ACKNOWLEDGEMENTS. We thank E. R. Unanue, Y.-X. Fu and H. Molina for discussions, and V. M. Holers and T. Kinoshita for complement receptor monoclonal antibodies. This work was supported in part by NIH grants (to D.D.C. and S.F.L.). D.D.C. is an investigator of the Howard Hughes Medical Institute.

CORRESPONDENCE and requests for materials should be addressed to D.D.C. (e-mail: chaplin@visar.wustl.edu).

'Anaphase' and cytokinesis in the absence of chromosomes

Dahong Zhang & R. Bruce Nicklas

Department of Zoology, Duke University, Durham, North Carolina 27708-1000, USA

ANAPHASE and cytokinesis are key processes in the segregation of replicated chromosomes to the daughter cells: in anaphase, chromosomes move apart; in cytokinesis, a cleavage furrow forms midway between the separated chromosomes. Some evidence suggests that chromosomes may be involved both in controlling the timing of anaphase onset^{1–3} and in dictating the position of the cleavage furrow³. Other evidence indicates that the controlling mechanisms are intrinsic to the spindle and the cell^{4–7}. Here we test these possibilities in grasshopper spermatocytes by observing spindles and cells after removal of chromosomes. We found that both anaphase and cytokinesis occur independently of chromosomes: stage-specific changes occur at an appropriate time and in the correct way, despite the absence of chromosomes. This finding is particularly noteworthy because chromosomes have an important impact on spindle microtubule assembly^{8,9} and the timing of anaphase onset¹⁰ in these cells.

Spermatocytes of the grasshoppers *Chortophaga australior* and *Melanoplus sanguinipes* have eleven bivalents and an X chromosome. Although chromosomes are required for spindle assembly in these cells⁹, once a spindle forms, the absence of chromosomes does not affect the integrity of the spindle⁵. We selected cells with a well-formed spindle and removed all the chromosomes by micromanipulation. We then followed spindle behaviour using video-enhanced polarization microscopy.

In cells from which all chromosomes were removed, the spindle changed with time much like a normal spindle with a normal set of chromosomes (Fig. 1a, b). At a stage approximating metaphase, a bipolar spindle was perfectly maintained (Fig. 1b, –112 and –14 min). Spindle microtubules, however, frequently grouped into bundles that either dispersed over time or elongated toward the cell equator and then shortened back to the poles (Fig. 2). At the time when normal cells entered anaphase, the cell devoid of chromosomes also progressed into 'anaphase' (Fig. 1a, b; 0 min). For cells lacking chromosomes, the onset of 'anaphase' was

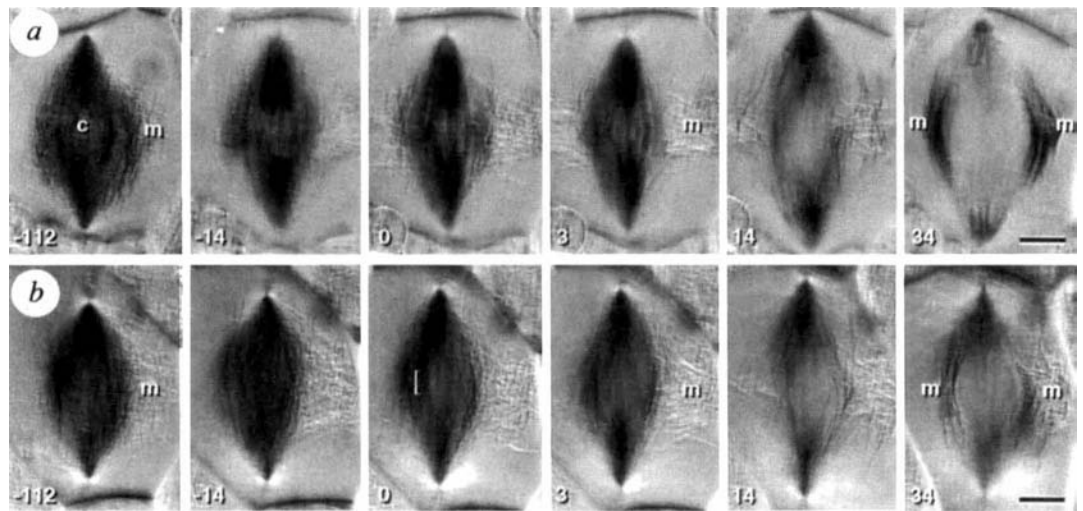
marked by the appearance of a gap in the spindle (Fig. 1b, bracket, 0 min), that is, by a much-reduced microtubule density at the equator. As anaphase progressed, this gap expanded toward the spindle poles (0 min onwards). Nine of ten cells without chromosomes entered 'anaphase' within 15 min of the time when anaphase commenced in control cells which had not been operated on. For ease of comparison of events in experimental and control cells, the onset of anaphase was set to 0 min (Figs 1 and 3). The rate of half-spindle shortening, determined by measurement of gap expansion in *Chortophaga australior*, averaged $0.47 \mu\text{m min}^{-1} \pm 0.11$ (95% confidence interval; $n = 5$), a rate similar to that for chromosome-to-pole movement (anaphase A) in a normal spindle ($0.58 \mu\text{m min}^{-1} \pm 0.05$; $n = 5$). Elongation of the spindle (anaphase B), so characteristic of normal anaphase, occurred on time in cells lacking chromosomes (Fig. 1a, b; 3–14 min) at a rate ($0.41 \mu\text{m min}^{-1} \pm 0.14$; $n = 4$) similar to that of the cells with chromosomes ($0.53 \mu\text{m min}^{-1} \pm 0.25$; $n = 4$; rate of spindle elongation is variable both in controls and cells without chromosomes). Finally, normal anaphase is marked by a redistribution of mitochondria (Fig. 1a, m), and an analogous process occurred on schedule in spindles without chromosomes (Fig. 1b).

Cytokinesis in the absence of chromosomes also appeared remarkably normal. At the time when control cells entered cytokinesis (Fig. 3a), a well defined cleavage furrow appeared in cells without chromosomes (Fig. 3b, arrow). The furrow was initiated precisely midway between the two spindle poles. Contraction of the furrow divided the cell into two cells without nuclei (Fig. 3b).

Our results agree with the early discoveries that echinoderm embryos can traverse the cell cycle repeatedly in the absence of nuclei^{1–6,11}. However, even though centrosomes duplicate during each cell cycle in these embryonic cells, a bipolar spindle does not appear⁵. In our experiments, we did not remove the chromosomes from the cell until a bipolar spindle had formed. Stage-specific changes in the spindles and the cells are revealed for the first time in the absence of chromosomes. We show that 'anaphase' and cytokinesis can occur normally and punctually in cells containing no chromosomes. This implies that some controlling mechanisms for these events are intrinsic to the spindle and the cell, but not the chromosomes. In the absence of chromosomes, the cell-cycle clock ticks on, staying precisely on time.

It has been suggested that ubiquitin-mediated destruction of a 'glue protein' that holds replicated chromosomes together may be needed to initiate anaphase^{1,2}. Although such a process probably

FIG. 1 All figures are polarization microscope images of *Chortophaga australior*. Anaphase in the presence of chromosomes. Microtubules are seen as black bundles. Time (min) is given in each panel, with 0 min corresponding to the initiation of anaphase. In metaphase (–112 and –14 min), chromosomes (c) align at the equator of the spindle, which is surrounded by mitochondria (m). Anaphase (0–34 min) is marked by separation of the chromosomes and spindle elongation (3–14 min) and by redistribution of the mitochondria. Initially, the mitochondria aggregate around the equator (compare –112 with 0–14 min) and later become aligned with the spindle axis (34 min). This unique mitochondrial redistribution serves as an additional marker for 'anaphase' when chromosomes are absent. *b*, 'Anaphase' in the absence of chromosomes. For ease of comparison, images in *a* and *b* at the same time intervals were selected. At a stage similar to metaphase (–112 and –14 min), spindle microtubule distribution was quite uniform. At the time when control cells entered anaphase, an abrupt decrease in microtubule mass at the equator (bracket) occurred (0–34 min) and mitochondria redistributed. Scale bars, 10 μ m. Grasshopper



spermatocytes from laboratory colonies of *Chortophaga australior* (REHN and HEBARD) or *Melanoplus sanguinipes* (FABRICIUS) were cultured as described⁸. Chromosomes were removed with a micromanipulation needle: each chromosome was detached from the spindle and then pulled out of the cell⁸. Video-enhanced polarization microscope images were acquired, processed and stored using an Image 1 system (Universal Imaging Corporation) and an optical disk recorder (model 3038, Panasonic Video Systems)⁸.

FIG. 2 Microtubule dynamics in the absence of chromosomes. Time is given in seconds. In the absence of chromosomes, spindle microtubules frequently bundled into thick fibres (f1, f2 and f3) that underwent elongation, shortening or dispersal. New fibres (f2 and f3) formed while an old one (f1) was disappearing. The half-life of these spindle fibre bundles was less than 1 min. Scale bar, 10 μ m.

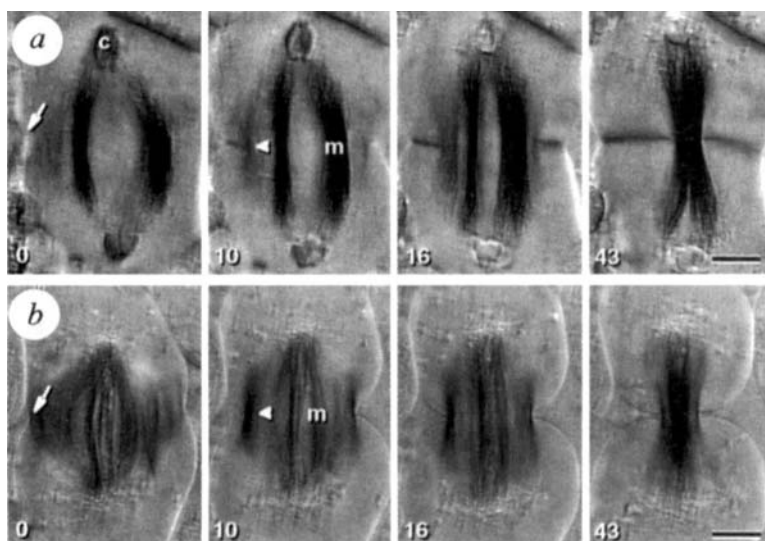
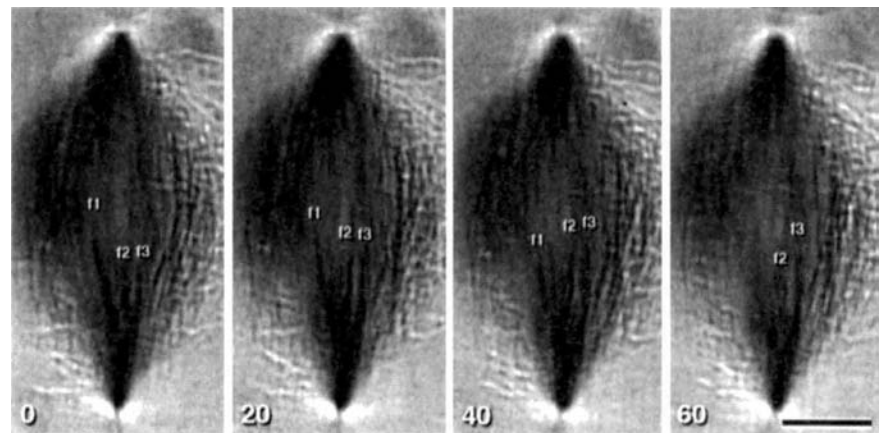


FIG. 3 Cytokinesis in the presence (a) and the absence (b) of chromosomes. A cleavage furrow (arrows) appeared midway between spindle poles regardless of the presence (a) or the absence (b) of chromosomes. Interzone microtubules (arrow heads) and mitochondria (m) were bundled together while the furrow gradually moved inward (10 min onwards). In the absence of chromosomes, the nuclear envelope did not reform. The final separation of daughter cells is not shown. Scale bars, 10 μ m.

occurs before the chromosomes can segregate, it is unlikely to be the trigger for anaphase onset: when the glue protein is removed along with the chromosomes, 'anaphase' is nevertheless initiated at the normal time (Fig. 1b). Evidently, something in addition to destruction of the glue protein (if and when it is present) controls the onset of anaphase.

In many cells, including grasshopper spermatocytes^{10,12-15}, anaphase is delayed in the presence of misaligned chromosomes by a cell-cycle checkpoint. However, as shown here, anaphase occurs on schedule even when there are no chromosomes for the checkpoint to check (Fig. 1b). Therefore, the checkpoint must detect misaligned chromosomes rather than counting the number of correctly aligned ones^{10,13,15}. This also underscores the proposition that a chemical message from the misaligned chromosomes is a negative signal to the checkpoint, preventing the onset of anaphase^{10,13,15}.

It has also been suggested that chromosomes dictate the position of the cleavage furrow in cytokinesis. This might occur, for example, if inner centromere proteins that dissociate from chromosomes at anaphase remain at the spindle equator and mark the furrow position³. Judging from our results, centromere proteins do not affect furrow position. The furrow occurs at the normal position when chromosomes are removed, taking the centromere proteins along with them, well before chromosomes align at the equator and well before centrosome proteins dissociate from the chromosomes.

Chromosome-to-pole movement in anaphase is coupled with the shortening of spindle microtubules¹⁶. Microtubule disassembly might 'drive' chromosomes to the spindle poles or, alternatively,

kinetochore motors might 'chew' microtubules as they drag chromosomes to the poles^{16,17}. Our observation that half-spindles without chromosomes shorten at a rate similar to that of normal spindle shortening, indirectly supports the idea that chromosomes, when present, might migrate to the poles merely by latching onto the ends of shortening microtubules. Further tests are needed, however, to discover whether microtubules in spindles devoid of chromosomes are actually shortening rather than being replaced by newly assembled, shorter microtubules. □

Received 21 March; accepted 3 June 1996.

- Holloway, S. L., Glotzer, M., King, R. W. & Murray, A. W. *Cell* **73**, 1393-1402 (1993).
- Luca, F. C. *Curr. Biol.* **3**, 716-718 (1993).
- Earnshaw, W. C., Bernat, R. L., Cooke, C. A. & Rothfield, N. F. *Cold Spring Harb. Symp. quant. Biol.* **56**, 675-685 (1991).
- Lorch, I. J. Q. *Jl microsc. Sci.* **93**, 475-486 (1952).
- Sluder, G., Miller, F. J. & Rieder, C. L. *J. Cell Biol.* **103**, 1873-1881 (1986).
- Sluder, G. & Rieder, C. L. *NATO ASI Series* **H72**, 211-224 (1993).
- Rappaport, R. *Int. Rev. Cytol.* **105**, 245-281 (1986).
- Zhang, D. & Nicklas, R. B. *J. Cell Biol.* **129**, 1287-1300 (1995).
- Zhang, D. & Nicklas, R. B. *J. Cell Biol.* **131**, 1125-1131 (1995).
- Li, X. & Nicklas, R. B. *Nature* **373**, 630-632 (1995).
- Wilson, E. B. *The Cell in Development and Heredity* 176-177 (Macmillan, New York, 1925).
- Zirkle, R. E. *Radiat. Res.* **41**, 516-537 (1970).
- McIntosh, J. R. *Cold Spring Harb. Symp. quant. Biol.* **56**, 613-619 (1991).
- Murray, A. W. *Nature* **359**, 599-604 (1992).
- Rieder, C. L., Schultz, A., Cole, R. & Sluder, G. *J. Cell Biol.* **127**, 1301-1310 (1994).
- Inoué, S. & Salmon, E. D. *Mol. Biol. Cell* **6**, 1619-1640 (1995).
- Coue, M., Lombillo, V. A. & McIntosh, J. R. *J. Cell Biol.* **112**, 1165-1175 (1991).

ACKNOWLEDGEMENTS. We thank A. McKibbins and S. Ward for technical support, and D. Maroni for editorial review. This investigation was supported in part by a Charles W. Hargitt Research Fellowship in Cell Biology from Duke University and by a grant from the Institute of General Medical Science, NIH.

CORRESPONDENCE and requests for materials should be addressed to D.Z. (e-mail: dzh@acupb.duke.edu).

Autocatalytic processing of the 20S proteasome

Erika Seemüller, Andrei Lupas & Wolfgang Baumeister

Max-Planck-Institut für Biochemie, D-82152 Martinsried bei München, Germany

THE Ntn (N-terminal nucleophile) hydrolases are enzymes with an unusual four-layer $\alpha + \beta$ fold¹⁻⁵. The amino-terminal residue (cysteine, serine or threonine) of the mature protein is the catalytic nucleophile⁶⁻¹⁰, and its side chain is activated for nucleophilic attack by transfer of its proton to the free N terminus², although other active-site residues may also be involved^{4,8}. The four currently known Ntn hydrolases (glutamine PRPP amidotransferase^{1,6}, penicillin acylase^{2,7}, the 20S proteasome^{3,8,9} and aspartylglucosaminidase^{4,10}) are encoded as inactive precursors, and are activated by cleavage of the peptide bond preceding the catalytic residue. It has been suggested that autocatalytic processing is a common feature of Ntn hydrolases, and proceeds by an intramolecular mechanism determined by their common fold⁵. Here we show that propeptide processing in the proteasome from *Thermoplasma acidophilum* is indeed autocatalytic, but is probably intermolecular. Processing is not required for assembly, is largely unaffected by propeptide length and sequence, and occurs before β -subunit folding is completed. Although serine is an acceptable active-site nucleophile for proteolysis, and cysteine for processing, only threonine is fully functional in both. This explains why threonine is universally conserved in active proteasome subunits.

The 20S proteasome is a ubiquitous, barrel-shaped protease^{11,12} formed by four seven-membered rings¹³ around a central cavity¹⁴. Although all subunits are structurally related, they can be divided into α - and β -type subunits¹⁵. The α -type subunits, which are proteolytically inactive¹⁶, form the outer rings, and the β -type

subunits, which contain the active site^{3,8}, form the inner rings of the complex¹⁷⁻¹⁹. Most, if not all, β -subunits are synthesized as inactive precursors and are processed during their incorporation into the proteasome^{15,19-23} (Fig. 1). In *Thermoplasma*, α -subunits are required for the processing and assembly of β -subunits¹⁶. Proteasomes differ in their level of complexity: eukaryotic proteasomes are formed by 14 different subunits (7 α - and 7 β -subunits)^{24,25}, whereas the archaeobacterial proteasome is formed by only two, α and β ²⁶.

To establish the autocatalytic nature of propeptide processing *in vitro*, separately purified α - and β -subunits were mixed, but no processing or assembly could be observed. Because β -subunits expressed in the absence of α -subunits have a tendency to precipitate, it was possible that they assume a misfolded state, which is incompetent for processing and assembly. A mixture of purified α - and β -subunits was therefore subjected to low-pH treatment (pH 2.6), which has been shown to disassemble and partly denature proteasomes²⁷. After dialysis at neutral pH, approximately 15% of β -subunits were recovered as part of assembled proteasomes. This shows that partly denatured β -subunits can revert to the native fold in the presence of α -subunits, which therefore not only chaperone the processing and assembly¹⁶, but also the folding of β -subunits. A third of the assembled β -subunits were processed, showing that the cleavage reaction is indeed autocatalytic. The high incidence of assembled but unprocessed β -subunits implies that assembly does not require processing; this is also the case *in vivo*, where all unprocessed mutants are nevertheless assembled efficiently into proteasomes. This is not altogether surprising because in eukaryotes the presumed inactive subunits are extended at their N terminus relative to active subunits (Fig. 1), and yet are incorporated efficiently into proteasomes. Although assembly does not require processing, processing does appear to be connected to assembly as, *in vitro*, no processed subunits remained unassembled. Two hypotheses can account for this observation: (1) processing occurs independently of assembly, but the free, processed subunits are preferentially incorporated into assembling complexes; or (2) the processing complex is a

TABLE 1 Processing and activity of β -subunit mutants

β -Subunit	Processing, (% of β -subunits)	Activity, (% of wild type)
Wild type β	100	100
Mutants of the prosequence		
$\beta\Delta$		100
$\beta\Delta$ Gly (-1)	70–80	80
$\beta\Delta$ Gly (-1) Ala	10–20	10
$^{\circ}\beta$	30–50	50
Mutants of Thr 1		
β Thr1Ser	40–60	50
$\beta\Delta$ Thr1Ser		100
β Thr1Ala	0	0
$\beta\Delta$ Thr1Ala		0
β Thr1Cys	≥ 95	0
$\beta\Delta$ Thr1Cys		0
β Thr1Cys/Lys33Ala	0	0
Mutants of Lys 33		
β Lys33Ala	0	0
$\beta\Delta$ Lys33Ala		0
β Lys33His	0	0
$\beta\Delta$ Lys33His		0
β Lys33Arg	0	0
$\beta\Delta$ Lys33Arg		0
Mutants of Glu 17		
β Glu17Asp	100	100
$\beta\Delta$ Glu17Asp		100
β Glu17Ala	0	0
$\beta\Delta$ Glu17Ala		0
Mutants of Asp 105		
β Asp105Ala	100	100
$\beta\Delta$ Asp105Ala		100
Mutants of Asp 166		
β Asp166Ala	0	0
$\beta\Delta$ Asp166Ala		0

Single amino-acid exchanges and deletion of the β -pro-region were obtained as described⁸. Two deletions of the pro-region were generated: $\beta\Delta$, lacking residues -7 to -1, and $\beta\Delta$ Gly (-1), lacking residues -7 to -2. In the mutant $^{\circ}\beta$, the entire pro-region is replaced by residues 1 to 34 of the α -subunit. For all mutants, the assembly into proteasome complexes was confirmed by native polyacrylamide gel electrophoresis (PAGE) and by electron microscopy. Complete proteasomes and separate subunits were purified from *Escherichia coli* BL21(DE3) by Ni-affinity chromatography using a genetically introduced, C-terminal tag of six histidines⁸. In all $\beta\Delta$ and $\beta\Delta$ Gly (-1) mutants, the initiator methionine was removed by the *E. coli* methionine aminopeptidase. Processing of the β -subunits was monitored by a band shift in SDS-PAGE and by N-terminal protein sequencing. Proteolytic activity of proteasomes was measured at 60 °C using Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin as described⁸.

TABLE 2 Intermolecular processing of inactive β -subunits by active subunits

Inactive subunit	Active subunit	Processing (%)	
		<i>in vitro</i>	<i>in vivo</i>
β Thr1Ala	$\beta\Delta$	<5	30–45
β Thr1Ala	$\beta\Delta$ Thr1Ser	<5	
β Lys33Ala	β		80–90
β Lys33Ala	$\beta\Delta$ Thr1Ser	5–10	30–45
β Lys33His	$\beta\Delta$ Thr1Ser	5–10	
β Lys33Arg	$\beta\Delta$ Thr1Ser	5–10	

In vitro, samples of assembled active and inactive proteasomes were disassembled and partly denatured in 0.1 M glycine buffer (pH 2.6), mixed and dialysed at room temperature for 10 h against TEN buffer (pH 7.5) as described²⁷. *In vivo*, active and inactive proteasome β -subunits were coexpressed in an operon structure containing sequentially the genes for the inactive β -, active β - and α -subunits. Both β -subunits contained C-terminal histidine tags and were purified by nickel-affinity chromatography⁸. The extent of processing was determined by N-terminal sequencing. Where no value is given, this was not determined.

before a subunit reaches its final folded structure. Because an intermolecular mechanism also requires a partly unfolded state, as the cleavage site is located at the bottom of the active-site cleft, it would seem that, either way, processing must occur before β -subunits reach their final fold.

In conclusion, the following factors are identified as important for processing, in addition to the essential role of α -subunits, which chaperone the processing, assembly¹⁶ and folding of β -subunits. (1) Sequence of the cleavage site. Gly(-1) and Thr 1 are entirely conserved in all active proteasome β -subunits, and mutation of either can markedly reduce the efficiency of processing. The rest of the prosequence is unconserved and can be deleted with only a minor effect on processing. Gly(-1) is important for recognition of the cleavage site, and Thr 1 is primarily important as the catalytic nucleophile. Although serine can substitute for Thr 1 in the proteolytic active site⁸, and cysteins can substitute for Thr 1 in the processing reaction, only threonine is an efficient nucleophile in both processes providing an explanation for its universal conservation. (2) Integrity of the active site. Except for β Thr1Cys, all mutations that abolish activity also prevent propeptide cleavage, indicating that processing involves residues which constitute the active site of the mature proteasome. (3) Geometry of active-site residues. Processing requires a different primary proton acceptor than proteolytic activity, because the amino group of Thr 1 is not free before cleavage of the propeptide. Also, cysteine can substitute for Thr 1 in the processing reaction but not in the mature active site, whereas serine can substitute in the mature active site but is inefficient in processing. Thus the geometry of the active site during the processing reaction is probably different to that in the mature proteasome. (4) Folding state. Assembled subunits cannot be processed further. Processing must therefore occur before assembly, and probably also before β -subunits have reached their final folded state. □

Received 8 February; accepted 22 May 1996.

- Smith, J. L. et al. *Science* **264**, 1427–1433 (1994).
- Duggleby, H. J. et al. *Nature* **373**, 264–268 (1995).
- Löwe, J. et al. *Science* **268**, 533–539 (1995).
- Ononen, C. et al. *Nature struct. Biol.* **2**, 1102–1108 (1995).
- Brannigan, J. E. et al. *Nature* **378**, 416–419 (1995).
- Soucié, J.-L., Hermodson, M. A. & Zalkin, H. J. *biol. Chem.* **263**, 3323–3327 (1988).
- Choi, K. S., Kim, J. A. & Kang, H. S. *J. Bact.* **174**, 6270–6276 (1992).
- Seemüller, E. et al. *Science* **268**, 579–582 (1995).
- Fenteany, G. et al. *Science* **268**, 726–731 (1995).
- Fisher, K. J. et al. *FEBS Lett.* **323**, 271–275 (1993).
- Lupas, A., Koster, A. J. & Baumeister, W. *Enzyme Prot.* **47**, 252–273 (1993).
- Peters, J.-M. *Trends biochem. Sci.* **19**, 377–382 (1994).
- Pühler, G. et al. *EMBO J.* **11**, 1607–1616 (1992).
- Hegerl, R. et al. *FEBS Lett.* **283**, 117–121 (1991).
- Zwickl, P. et al. *Biochemistry* **31**, 964–972 (1992).
- Zwickl, P., Klein, J. & Baumeister, W. *Nature struct. Biol.* **1**, 765–770 (1994).
- Grziwa, A. et al. *FEBS Lett.* **290**, 186–190 (1991).
- Kopp, F., Dahlmann, B. & Hendil, K. J. *molec. Biol.* **229**, 14–19 (1993).
- Schauer, T. M. et al. *J. struct. Biol.* **111**, 135–147 (1993).
- Tamura, A. et al. *Curr. Biol.* **5**, 766–774 (1995).
- Lilley, K. A., Davison, M. D. & Rivett, A. J. *FEBS Lett.* **262**, 327–329 (1990).
- Lee, L. W. et al. *Biochim. biophys. Acta* **1037**, 178–185 (1990).
- Chen, P. & Hochstrasser, M. *EMBO J.* **14**, 2620–2630 (1995).
- Hilt, W. & Wolf, D. H. *Molec. Biol. Rep.* **21**, 3–10 (1995).
- Tanaka, K. *Molec. Biol. Rep.* **21**, 21–26 (1995).
- Dahlmann, B. et al. *FEBS Lett.* **251**, 125–131 (1989).
- Grziwa, A. et al. *Eur. J. Biochem.* **233**, 1061–1067 (1994).
- Guan, C. et al. *J. biol. Chem.* **271**, 1732–1737 (1996).

ACKNOWLEDGEMENTS. We thank F. Lottspeich and J. Kellermann for protein sequencing; U. Santarius for electron microscopy; and M. Boicu for DNA sequencing.

CORRESPONDENCE and requests for materials should be addressed to W.B.

Visualization of ordered genomic RNA and localization of transcriptional complexes in rotavirus

B. V. Venkataram Prasad*, R. Rothnagel*, C. Q.-Y. Zeng†, J. Jakana*, J. A. Lawton‡, W. Chiu* & M. K. Estes†

*Verna and Marrs McLean Department of Biochemistry, and the W. M. Keck Center for Computational Biology, †Division of Molecular Virology, and ‡Program in Cell and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA

IN double-stranded-RNA (dsRNA) viruses found in animals¹, bacteria² and yeast³, the genome is transcribed within the structurally intact core of the virion with extraordinary efficiency. The structural organization of the genome and the enzymes involved in the transcription inside any of these viruses, critical for understanding this process, is not known. Here we report what we believe is the first three-dimensional characterization of the viral genome and the transcription complex in a prototypical dsRNA virus. Rotavirus is a large (diameter 1,000 Å) icosahedral virus composed of three capsid protein layers and 11 dsRNA segments⁴⁻⁹. It is the most important cause of gastroenteritis in children, accounting for over a million deaths annually¹⁰. We show that viral dsRNA forms a dodecahedral structure in which the RNA double helices, interacting closely with the inner capsid layer, are packed around the enzyme complex located at the icosahedral 5-fold axes. The ordered RNA accounts for about 4,500 out of a total 18,525 base pairs in the genome, the largest amount of icosahedrally ordered RNA observed in any virus structure to date. We propose that the observed organization of the dsRNA is conducive for an orchestrated movement of the RNA relative to the enzyme complex during transcription.

Genome transcription in rotavirus occurs within double-layered particles (DLPs) following the removal of the outer capsid protein layer at cell entry¹¹. The structural integrity of the DLP, which is composed of the viral proteins VP1, VP2, VP3 and VP6, is essential for transcription. Although the overall structure of the rotavirus has been described⁵⁻⁹, structural details of the genome organization and the locations of the internal proteins VP1, the viral polymerase¹², and VP3, the guanyl transferase¹³, have not, to our knowledge. We have now determined the three-dimensional structure of transcriptionally competent DLPs to a resolution of 19 Å using a 400-kV electron cryomicroscope, which enabled features inside the virus to be observed more clearly than previously (Fig. 1). To identify the internal features in terms of protein and RNA, we carried out difference imaging between native DLPs and various recombinant baculovirus-expressed virus-like particle (VLPs) using a 100-kV electron cryomicroscope at a resolution of 23 Å. We used VLPs composed of VP2 alone (VLP-2), VP2 and VP6 (VLP-2/6), and VP1, VP2, VP3 and VP6 (VLP-1/2/3/6).

Comparison of the radial-density profiles computed from the structures of the DLP and VLPs (Fig. 2) indicates that VP2 lies between 230 and 260 Å radius, consistent with earlier results⁹, and that the VP6 layer is between 260 and 350 Å. The radial-density profile of the DLP has significant mass density inside 230 Å, whereas in the VLPs there is little significant mass inside this radius. The VP2 surface in all the structures appears similar but for a more prominent dimple at the 5-fold axes in the DLP structure (Fig. 3, top row). Although most of the VP2 lies between the radii 235 and 260 Å, forming a relatively smooth, contiguous

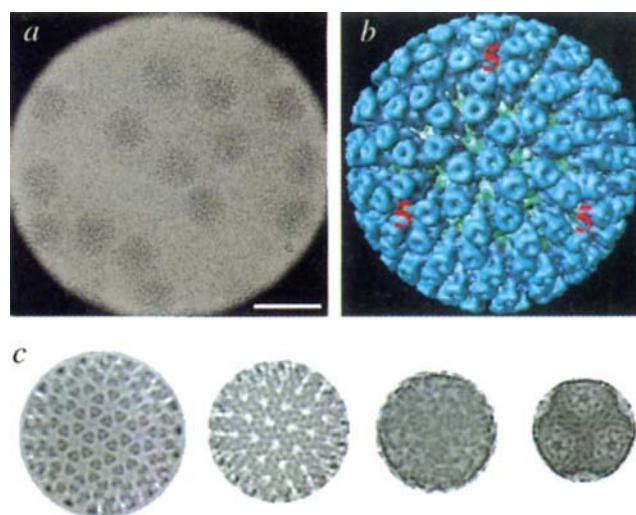


FIG. 1 *a*, Electron cryomicrograph of rotavirus DLPs (diameter, 700 Å) embedded in a thin layer of vitreous ice. Scale, 1,000 Å. *b*, Surface representation, depth cued in colour (colour chart in Fig. 3), of the 3-dimensional structure of the DLP at a nominal resolution of 19 Å viewed along the icosahedral 3-fold axis. The 5-fold positions on one of the icosahedral facets are shown. A feature not seen in previous lower-resolution reconstructions is the hole at the centre of each VP6 trimer. *c*, Projections of radial sections, viewed along the icosahedral 3-fold axis, computed from the DLP structure. Mass density included is between the radii (from left to right): 300–350 Å, 260–300 Å, 235–260 Å, and 200–235 Å. These projections offer a means, unbiased by choice of contour level, to examine the mass-density distribution at internal radii. Between 200 and 235 Å radius, an interesting feature is the strands of mass density running between the neighbouring icosahedral 3-fold axes surrounding a star-shaped mass density at the 5-fold axis.

METHODS. Native DLPs were purified from rotavirus-infected MA104 cells as before⁵. The specimen was embedded in a thin layer of vitreous ice on holey carbon films using standard procedures²². Electron cryomicroscopy was carried out on a 400-kV electron microscope (JEOL 4000) using spot-scan procedures²³ at -168°C . Images were recorded using electron doses of $4\text{--}5\text{ e}^{-}\text{ per } \text{\AA}^2$ at a magnification of $\times 30,000$. The chosen electron micrographs were digitized with a scanning interval of $5.33\text{ \AA}$ in the object. Orientations of the particles, using common lines²⁵ were determined as described^{26,27}. Three-dimensional image reconstruction was computed using cylindrical expansion methods²⁴, with 127 particles having unique orientations to a nominal resolution of 19 Å. In the final map, full icosahedral symmetry was imposed by real-space 3-fold averaging. The defocus and resolution were assessed according to criteria described in ref. 26. The defocus value of $1.8\text{ }\mu\text{m}$ was determined from the contrast transfer function ring positions in the sum of particle Fourier transforms. As all the data used in the reconstruction were within the first zero of the contrast transfer function of the microscope for this defocus, no correction was made for the effects of the contrast transfer function.

shell, a small portion of VP2 extends further inward at the 5-fold axes to form a pentagonal structure (Fig. 3, middle row).

Attached to the inside tip of VP2, at the 5-fold axis, is a flower-shaped structure in the VLP-1/2/3/6 structure (arrow in Fig. 3, bottom row) extending inward to a radius of 160 Å. The fact that this feature is reproducible in independent reconstructions assures its reliability and precludes the possibility that it is a result of 5-fold symmetrization in the reconstruction. The presence of this feature in VLP-1/2/3/6 and its absence in the VLP-2/6 structure provides strong evidence that it is composed of VP1 and/or VP3. Preliminary structural analysis of VLP-1/2/6 and VLP-2/3/6 particles also supports the idea that this flower-shaped structure represents a complex of VP1 and VP3 (data not shown). The total mass of each flower-shaped structure accounts for about 25% of the expected mass of a complex of VP1 (M_r 125,000) and VP3 (M_r 98,000). It is possible that remaining portions of VP1 and VP3

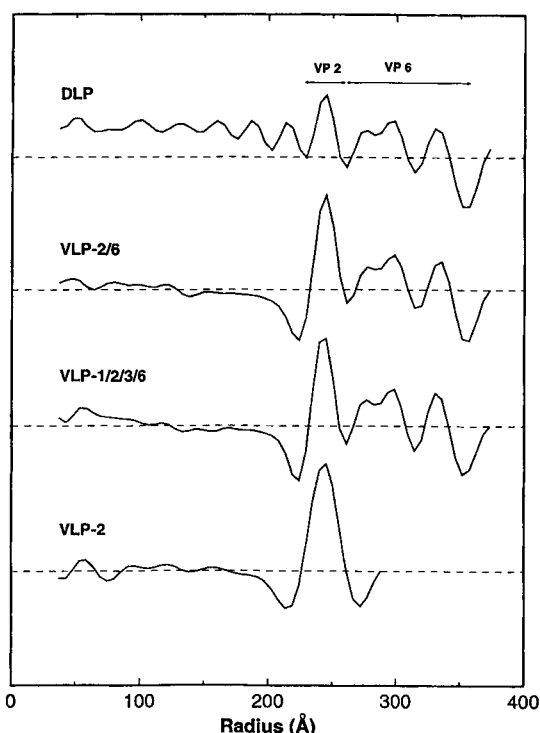


FIG. 2 Radial-density profiles computed from the three-dimensional density maps of the DLP and various VLPs.

METHODS. Recombinant VLPs were obtained from baculovirus-infected Sf9 cells as described^{16,28,29}. The three-dimensional structures were determined using cryo images recorded on a JEOL 1200 microscope using 100-kV electrons. Each of the VLPs was imaged together with DLPs to account for any changes in magnification during microscopy. Images were recorded at 1.1 μm underfocus, with an electron dose of $\sim 5 \text{ e}^- \text{ per } \text{\AA}^2$ at a magnification of $\times 30,000$. Three-dimensional structural analyses of DLP, VLP-2/6 and VLP-1/2/3/6 were carried out using 105, 93 and 101 particles, respectively, to a nominal resolution of 23 $\text{\AA}$; VLP-2 could only be resolved to 30 $\text{\AA}$ as it was difficult to obtain high concentrations of these particles¹⁶. The VLP-2 structure was used only to confirm the radial location of the VP2 layer. The radial locations of VP6 and VP2 are indicated.

extend further inside the radius of 160 $\text{\AA}$ and are transparent to our structural analysis because the virus structure lacks icosahedral symmetry below this radius. Thus the flower-shaped structure may represent the structurally discernible portion of the VP1–VP3 enzyme complex. Both the location of this complex as well as mass calculations indicate that there are 12 molecules of VP1 and VP3 per virion, consistent with biochemical analysis¹⁴. However, because of our reconstruction procedures, which implicitly use icosahedral symmetry, we cannot exclude the possibility that fewer than 12 molecules of VP1 and VP3 are present.

At a radius of 230 $\text{\AA}$ in the DLP structure, a strong mass density surrounding the 5-fold axes and between the neighbouring 3-fold axes is clearly seen (star in Fig. 3, middle row). The complete absence of this mass density in the VLP-2/6 and the VLP-1/2/3/6 structures strongly implies that this additional mass in the DLP is due to the genomic RNA. Structural analysis of the DLP at 19 $\text{\AA}$ resolution using a 400-kV electron cryomicroscope resolves this region more clearly as parallel strands of tube-like mass density that form a dodecahedral shell when viewed at a slightly higher contour level (Fig. 4). These tubes of mass density have an average diameter of 20 $\text{\AA}$, typical of a dsRNA double helix, lending further support to our interpretation. These strands of RNA encircle the VP1–VP3 complex (shades of red in Fig. 4, left). We should point out that the icosahedral packing of RNA does not imply 60-fold repetition in the gene sequence, but indicates that portions of

the dsRNA occupy an icosahedrally equivalent volume in the structure.

Why is the RNA icosahedrally ordered? VP2 is known to have RNA-binding activity¹⁵. It is clear from Fig. 3 that there are several points of contact between the inwardly protruding portions of VP2 and the RNA surrounding each 5-fold axis. Examination of the structure at various contour levels suggests that weaker interactions may also occur across the icosahedral 2-fold axes. Thus VP2, which is icosahedrally assembled, induces icosahedral ordering on closely interacting portions of the RNA. Icosahedral ordering of RNA does not seem to be maintained below a radius of 190 $\text{\AA}$. Another question is why the structural integrity of the DLP is necessary for transcription. It appears that the VP2 protein has a predominantly scaffolding role in the transcription process, whereas VP1 and VP3 have enzymatic functions. The inner surface of the VP2 layer not only provides a structural support for the RNA, but also helps to position the transcription complex properly. The outer surface of the VP2 layer provides a structural platform for the assembly of VP6 trimers, preventing aggregation of the core particles (VP2, VP1, VP3 and the genome) which are known to be highly hydrophobic¹⁶. Thus efficient transcription requires the structural integrity of the DLP.

Our structural analysis represents the first visualization of an ordered genome in a large, complex dsRNA virus. Icosahedrally ordered nucleic acid has been previously observed in small virus

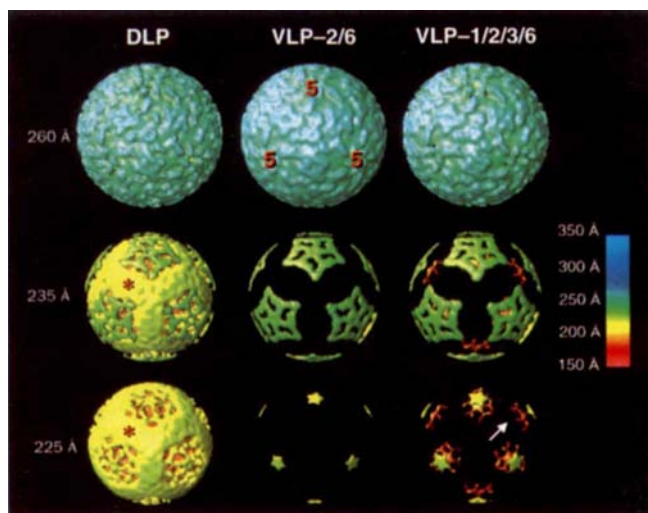
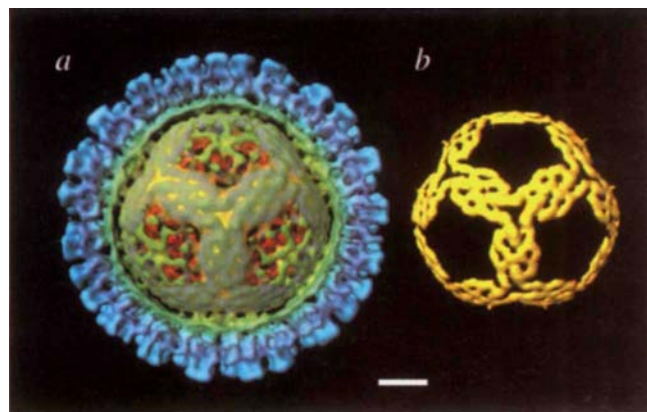


FIG. 3 Comparison of the internal features in the 23 $\text{\AA}$ structures of DLP, VLP-2/6 and VLP-1/2/3/6. All the surface representations are radially depth-cued according to the colour chart and are viewed along the icosahedral 3-fold axis. These radial cutaways are computed from a radius of 160 $\text{\AA}$ to the various radii, as indicated. The structural features between the radii 260 and 350 $\text{\AA}$ are invariant between these structures and are not shown. The contour level in these representations for each structure is chosen such that the mass between the radius of 260 and 350 $\text{\AA}$ accounts for ~ 780 molecules of VP6 (M_r 44,800), assuming a density of 1.30 g cm^{-3} . RNA density (yellow; indicated by stars) begins to appear in the structure of the DLP at a radius of 230 $\text{\AA}$; corresponding regions in the VLP-2/6 and VLP-1/2/3/6 are empty. VP2 protrudes inwards at the 5-fold axes to a radius of ~ 220 $\text{\AA}$. Between the radii 160 and 220 $\text{\AA}$, a flower-shaped mass density (arrow) is seen attached to the VP2 tips at the 5-fold axes in the VLP-1/2/3/6 structure. A similar density distribution is seen at the corresponding places in the DLP structure. The flower-shaped density was also seen in reconstructions in which only 5-2 symmetry was used, but this feature was more obvious when the full 5-3-2 symmetry was used for averaging. Structural features below 160 $\text{\AA}$ radius are not shown as they vary significantly between independent reconstructions, suggesting a lack of icosahedral symmetry below this radius.

FIG. 4 Structural organization of RNA inside rotavirus. Left, cutaway from the 19 Å structure of the rotavirus DLP, showing internal organization. Surrounding the surface representation at a radius of 235 Å, a thin cross-section of the mass density between 235 and 350 Å representing the VP2 and VP6 layers is shown for reference. The radial colouring is the same in all figures. VP2 (between 230 and 260 Å) is represented in shades of green and VP6 (between 260 and 350 Å radius) in shades of blue. The VP1–VP3 complex (between radii 160 and 200 Å; see also Fig. 3) at the 5-fold axes is shown in red. Portions of VP2 are seen at the 5-fold axes in shades of green. Because of the differences in the densities between the protein ($\sim 1.30 \text{ g cm}^{-3}$) and dsRNA ($\sim 1.61 \text{ g cm}^{-3}$)³⁰, different contour levels have to be used to represent the RNA. The mass density at a slightly higher contour level ($\sim 1.5\sigma$ level from the mean in the three-dimensional map), which represents dsRNA strands, is shown in grey inside the yellow regions (compare this region with that in the DLP structure in Fig. 3, second row). Right, the dodecahedral shell of the ordered RNA, extracted from the 19 Å structure, shown separately for clarity. Each strand is about 20 Å in diameter. The strands are separated from each other by ~ 25 –30 Å. The volume occupied by the ordered RNA, assuming a density of 1.61 g cm^{-3} , accounts for about 25% of the total mass ($M_r \sim 10^7$) of the genome. This value translates to $\sim 4,500$ out of a total of 18,525 base pairs⁴. Scale bar, 100 Å.



structures by X-ray crystallography^{17–21}. The maximum number of base pairs visualized in a virus structure is ~ 300 (ref. 20). In our structure, ordered RNA accounts for about $\sim 4,500$ base pairs. This extensive ordering of the genome may be critical for the endogenous transcriptase activity, perhaps facilitating an orchestrated movement of the genome through the enzyme complex. This motion could be driven by the continuous exit of newly synthesized mRNA. The pores surrounding the 5-fold axes, in the VP2 layer, are large enough to allow the messenger RNA to leave, although it is also possible that VP2 may undergo conformational

changes during transcription to facilitate the exit of mRNA. Our understanding of this process will improve by observing changes in the RNA and viral proteins during active transcription and under conditions in which the DLP is transcriptionally incompetent. Although it is not possible to determine the positions of different segments of dsRNA in the structure, it is possible that a substantial portion of each of the 11 segments could be ordered around a 5-fold axis, with each segment interacting with a VP1–VP3 complex. □

Received 28 March; accepted 12 June 1996.

- Fields, B. N. in *Virology* (eds Fields, B. N. et al.) 1553–1555 (Raven, New York, 1996).
- Gottlieb, P., Strassman, J., Qiao, X., Frucht, A. & Mindich, L. J. *Bact.* **172**, 5774–5782 (1990).
- Wickner, R. B. *J. biol. Chem.* **268**, 3797–3800 (1993).
- Estes, M. K. in *Virology* (eds Fields, B. N. et al.) 1625–1655 (Raven, New York, 1996).
- Prasad, B. V. V., Wang, G. J., Clerx, J. P. M. & Chiu, W. J. *molec. Biol.* **199**, 269–275 (1988).
- Prasad, B. V. V., Burns, J. W., Marietta, E., Estes, M. K. & Chiu, W. *Nature* **343**, 476–478 (1990).
- Shaw, A. L. et al. *Cell* **74**, 693–701 (1993).
- Yeager, M., Berriman, J. A., Baker, T. S. & Bellamy, A. R. *EMBO J.* **13**, 1011–1018 (1994).
- Prasad, B. V. V. & Chiu, W. in *Rotaviruses* (ed. Ramig, R.) 9–29 (Springer, Berlin, 1994).
- Kapikian, A. Z. & Chanock, R. M. in *Virology* (eds Fields, B. N. et al.) 1657–1708 (Raven, New York, 1996).
- Cohen, J. J. *gen. Virol.* **36**, 395–402 (1977).
- Valenzuela, S. et al. *J. Virol.* **65**, 3964–3967 (1991).
- Liu, M., Mattion, N. M. & Estes, M. K. *Virology* **188**, 77–84 (1992).
- Liu, M., Offit, P. A. & Estes, M. K. *Virology* **163**, 26–32 (1988).
- Labbe, M., Baudoux, P., Charpillienne, D., Poncet, D. & Cohen, J. J. *gen. Virol.* **75**, 3423–3430 (1994).
- Zeng, C. Q.-Y. et al. *Virology* **201**, 55–65 (1995).
- Chen, Z. et al. *Nature* **245**, 154–159 (1989).

- Tsao, J. et al. *Science* **251**, 1456–1464 (1991).
- Mckenna, R. et al. *Nature* **355**, 137–143 (1992).
- Fisher, A. J. & Johnson, J. E. *Nature* **361**, 176–179 (1993).
- Larson, S. B. et al. *Nature* **361**, 179–182 (1993).
- Dubochet, J. et al. *Q. Rev. Biophys.* **21**, 129–228 (1988).
- Brink, J., Chiu, W. & Dougherty, M. *Ultramicroscopy* **46**, 229–240 (1992).
- Crowther, R. A. *Phil. Trans. R. Soc. Lond. B* **261**, 221–230 (1971).
- Fuller, S. D. *Cell* **48**, 923–934 (1987).
- Zhou, Z. H., Prasad, B. V. V., Jakana, J., Rixon, F. J. & Chiu, W. J. *molec. Biol.* **242**, 456–469 (1994).
- Lawton, J. A. & Prasad, B. V. V. *J. struct. Biol.* **116**, 209–215 (1996).
- Crawford, S. E. et al. *J. Virol.* **68**, 5945–5952 (1994).
- Zeng, C. Q.-Y., Wentz, M. J., Cohen, J., Estes, M. J. & Ramig, R. F. *J. Virol.* **70**, 2736–2742 (1996).
- Shatkin, A. J. & Sipe, J. D. *Proc. natn. Acad. Sci. U.S.A.* **59**, 247–253 (1968).

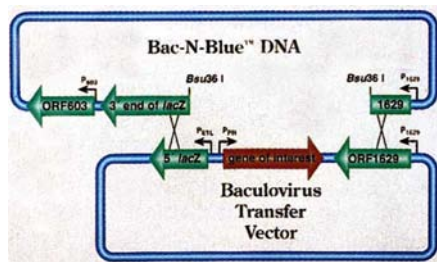
ACKNOWLEDGEMENTS. We thank R. F. Ramig and M. F. Schmid for comments on the manuscript. This work has been supported by grants to B.V.V.P., M.K.E. and W.C. from the NIH, the NSF, and the W. M. Keck Foundation. J.A.L. acknowledges a training fellowship from the NIH.

CORRESPONDENCE and requests for materials should be addressed to B.V.V.P. (e-mail: vprasad@bcm.tmc.edu).

Molecular breakdown

This week's tender covers DNA technology, including a kit for DNA analysis using high-resolution gel electrophoresis, a hybridization and detection system, primer design software and a purification kit for high-molecular-weight genomic DNA.

Invitrogen has inaugurated its new **high-efficiency AcMNPV baculovirus DNA** for use with the baculovirus expression system. Bac-N-Blue DNA yields a stated 80 per cent or greater recombinant virus, reducing the time necessary to obtain a pure recombinant to as little as 14 days. Bac-N-Blue DNA can be used with any



Invitrogen's AcMNPV baculovirus DNA.

polyhedrin promoter-based baculovirus transfer vector, including the company's pBlueBac vectors. Recombination with a pBlueBac vector will produce blue recombinant plaques that are easy to screen and thus should save time. Insectin liposomes are included for maximum transfection efficiency.

Reader Enquiry No. 100

Detection and analysis

Enzo Diagnostics has announced the availability of its new MaxSens **hybridization and detection systems**. This complete line of products for membrane hybridization and detection has been optimized to provide improved hybridization and washing conditions for background-free, high-sensitivity results with non-radioactive probes that have been labelled with biotin, digoxigenin or fluorescein haptens. The system is designed for use with DNA and RNA probes prepared by nick translation, random-primed or *in vitro* RNA-transcript labelling procedures. It is specifically for use with oligonucleotide probes prepared by terminal labelling procedures. The company has developed a unified format for membrane hybridization and detection, regardless of which hapten label is used. Each kit contains reagents, including hybridization membranes and a detailed protocol for hybridizing ten blots. The hybridization systems are optimized for use with one of six different MaxSense detection systems — either alkaline phosphatase- or horseradish peroxidase-linked

detection reagents for biotin, digoxigenin and fluorescein haptens. This procedural unification should make it straightforward to do multiple detections on a single blot. Detection of biotin can be combined simply and easily with detection of fluorescein or digoxigenin. Use of detection reagents linked with different colour-generating enzymes quickly yields two hybridization results.

Reader Enquiry No. 101

Bio Image now offers new **software for analysing high-density colony or DNA YAC libraries**. The HDG Analyser software provides accurate spot detection and quantification for all types of grid arrays (4 × 4, 6 × 6, etc.). It compensates for membrane deformation with flexible, easy-to-use triangles, which are said to be an improvement over rigid grids. Manual analysis of colony and DNA YAC libraries is often time-consuming and prone to error. The software supports both qualitative (screening) and quantitative (expression analysis) applications. The software is available for use with Sun SPARCstations.

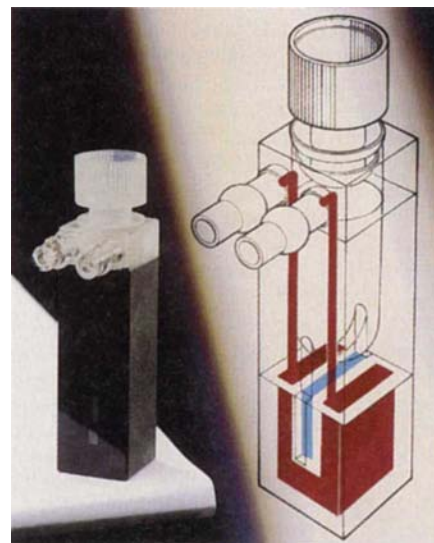
Reader Enquiry No. 102

Pharmacia Biotech has introduced a **DNA analysis kit** that brings increased convenience to high-resolution gel electrophoresis. Convenience and speed are said to be key features with the ExcelGel DNA analysis kit, which contains pre-cast, ready-to-run polyacrylamide gels and buffer strips. The resolution is said to exceed that which is possible using agarose electrophoresis, down to 8 bp in the 100- to 200-bp range. The kit is also said to improve consistency by avoiding the problems associated with casting and running home-made acrylamide gels. The kit can be used for many modern DNA analysis techniques that require high resolution, such as RAPD, SSCP, ARDRA and VNTR. ExcelGel is a wet, pre-cast polyacrylamide gel that has 48 preformed sample wells to facilitate fast loading of samples. The problems of handling toxic chemicals and numerous solutions used in casting and running a home-made gel are thus avoided. After about 80 min of non-denaturing separation on the Multiphor II flatbed electrophoresis system, the gel can be non-radioactively stained using the PlusOne silver staining kit. The kit is said to produce sharp bands, which allow the straightforward visualization of the results.

DNA bands may be directly cut out from the gel for amplification and other DNA analysis purposes, such as sequencing. The gels can also be dried for documentation, which conserves the DNA bands for permanent storage and future analysis.

Reader Enquiry No. 103

Optiglass has recently introduced a new version of the Starna Brand **sub-micro cell**, which features continuous temperature control. The cell is available with two sizes of sample chamber (100 µl and 160 µl). It has a separate chamber that surrounds the sample chamber and through which a temperature-controlled liquid, such as silicone oil, may be circulated. This allows the sample to be heated



Starna sub-micro cells from Optiglass.

or cooled to the required temperature and for it to be maintained at that level. This cell is particularly useful for DNA analysis, where temperature control is very important.

Reader Enquiry No. 104

PrimeScreen allows researchers to conveniently **test for human or rat gene expression levels of cytokines, chemokines, regulatory enzymes and enzymes involved in immune-response modulation**. A key application involves *in vivo* or *in vitro* testing for upregulated gene messages derived from whole tissue or cultured cells. PrimeScreen panels are designed to save time over conventional northern hybridization methods by using an RT-

PRODUCT REVIEW

PCR-based method of gene expression analysis. Results are possible in as little as three hours without the use of radioactive isotopes. Each panel contains three purified synthetic oligonucleotide primer pairs sufficient for 50 determinations. All primer pairs are optimized for identical PCR cycling conditions. Panels are designed to probe for upregulated gene expression of three genes grouped by functional activity. Also included in each panel is a β -actin primer pair to be used alone as an internal control, and in combination with the β -actin cDNA component of the kit as an external positive control.

Reader Enquiry No. 105

Software/Database

A new **primer design program** called Primer Premier 4.0 is now available for Power Macintosh and Window 3.1, NT and Windows95. The program can design primers automatically or with full manual control for applications such as PCR, sequencing or hybridization. From the selected pool of primers, mutually compatible nested/multiplex primers or pairs can be chosen. Its features include an editing facility (where any primer can be mutated), full degeneracy, comprehensive analysis of secondary structures, as well as a table of properties (including the melting temperature calculated using nearest neighbour theory and optimal annealing for each primer). The selected primers can be managed with the database storage facility. The program even makes the primer synthesis process easier to use by automatically creating an order form addressed to the vendor of your choice. Restriction enzyme analysis is available with multiple output formats: table, annotated sequence or map. Functions to edit the sequence and to translate it using standard or any mitochondrial organelle codon table are provided. Graphs of melting temperature, internal stability and free energy are available on-line, as well as in a printed format.

Reader Enquiry No. 106

ADVERTISEMENT

OLIGONUCLEOTIDES

£1 per base

- stringent quality control
- express turnaround
- column purified: ready to use

By research labs for research labs

Molecular Biology Services

King's College School of Medicine and Dentistry
Rayne Institute, 123 Coldharbour Lane, London SE5 9NU
Tel: 0171-346 3126 Fax: 0171-733 3877

READER ENQUIRY NO. 10

IntelliGenetics has unveiled a new service for its popular **Geneseq database of nucleic acid and protein sequences**. Subscribers can exchange the database updates they receive quarterly on magnetic tape for the ability to download updates of Geneseq-NA and Geneseq-AA every two weeks via 'file-transfer protocol' (ftp) over the Internet. Geneseq is a complete source of patent information that scientists can use to verify that sequences central to their work have not been patented by other researchers anywhere in the world. IntelliGenetics is also a supplier and a developer of bioinformatics software, high-performance database searching tools and value-added sequence databases. The group also undertakes contract research for pharmaceutical drug discovery and design.

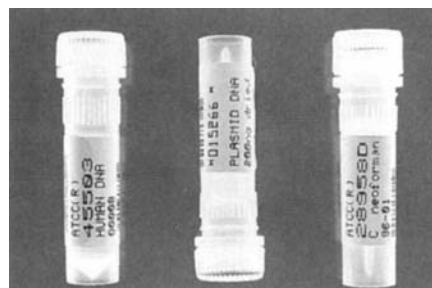
Reader Enquiry No. 107

Molecular means

Nunc's **replication system** consists of products designed for the company's OmniTray and 384-well plate. These products can be used for replication of recombinant DNA libraries, inoculation of filters for colony hybridization, phage typing and several other applications. Pall Biotyne B nylon membranes are precut to fit correctly into Nunc's OmniTray for manual or automated arraying, which can be performed directly on a solid support. Nunc 384 and 96-pin replicators are well suited to transferring small volumes of inoculum from MicroWell plates to other plates or solid supports. Neither repeated autoclaving, nor flame sterilization, will alter the performance or the precision alignment of the replicator's stainless steel pins. The MicroWell plate and OmniTray copiers are simple registration devices for Nunc replicators. The machined polypropylene has a unique set of alignment holes used to guide the replicator pins. They precisely register the pins to agar, hybridization membranes or the wells of MicroWell plates.

Reader Enquiry No. 108

ATCC is offering **genomic DNA preparations from several microbial strains and cell lines**. Scientific researchers can now purchase genomic DNA from 11 yeast strains in 25- μ g aliquots, plasmid DNA from ten molecularly cloned viruses in 2- μ g aliquots and genomic DNA from four human tumour cell lines and from two sets of paired normal human/tumour cells in 25-pg and 100-pg aliquots. Each pair of human cell lines was derived from the same individual, permitting a comparison



Genomic DNA from microbial sources.

of normal (EBV-transformed B lymphocytes) and tumour cells. The high-molecular weight DNA is isolated under aseptic conditions to prevent cross-contamination with extraneous DNA. The purified DNA is evaluated for integrity, purity and quality by several methods, such as agarose gel electrophoresis, spectrophotometry, restriction digestion and suitability for amplification by PCR.

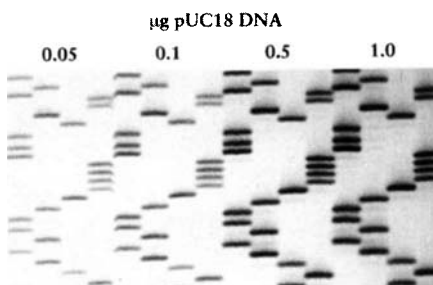
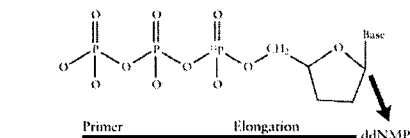
Reader Enquiry No. 109

A new **optical biosensor system**, specially designed for higher throughput analysis of biomolecular interaction, has been produced by Affinity Sensors. IAsys Auto+ offers all the advantages of the company's stirred micro-cuvette system, plus a range of features giving new and significant benefits to users. They include the choice of fully or semi-automated operation and simultaneous analysis of two channels for higher throughput. Using the 'adaptive sequence kontrol' (ASK) capability, the new biosensor allows method programs to include data interrogation, simple mathematical formulae and write if/then commands. Alternatively, immediate hands-on operation of the instrument and direct control of the autosampler is provided by the image-based software known as 'point and transfer' (PAT). IAsys Auto+ uses a biosensor with two vibro-stirred micro-cuvette wells to allow simultaneous experiments using different ligands, analyses and chemistries, with no risk of cross-contamination. The design is said to offer time savings and flexibility by providing increased sample throughput, as well as duplicate or referenced assays. Direct pipetting into the micro-cuvette wells also minimizes sample usage and avoids the risk of blockage presented by complex fluids. No sample preparation is required. Moreover, the micro-cuvettes are available with a choice of derivatized sensor surfaces. Combined with well-established coupling procedures, they give maximum versatility in the choice of ligand immobilization strategy for a wide range of molecule types. The system uses Windows-based software for easy operation, results analysis and report generation. The company's FASTfit data analysis program provides

an intuitive interface for analysing binding and dissociation experiments and for calculating rate and binding constants.

Reader Enquiry No. 110

Amersham's terminator **cycle sequencing kit** now combines Redivue ^{33}P -labelled dideoxynucleotide (ddNTP) terminators with Thermo Sequenase. This is said to allow difficult sequencing tasks, such as heterozygote analysis, to be performed routinely. According to the manufacturer, the new terminators are more efficient than other labelled nucleotides, as they will specifically label only properly terminated DNA chains. Prematurely terminated chains are not labelled, 'stop' artefacts (for example, BAFLs) and most background bands are eliminated. Furthermore, by removing stop artefacts, routine use of dITP is possible enabling a reduction of strong compression artefacts. These features, coupled with the fact that the label is ^{33}P , should lead to cleaner, more reliable and easier to read data. Mixed population DNA sequences, such as those found in heterozygotes, are often difficult to interpret because of background and uneven bands on conventional cycle-sequencing gels. The use of Thermo Sequenase helps overcome this problem because its efficient incorporation of ddNTPs produces more even bands. Moreover, the use of ^{33}P termina-



Amersham's ^{33}P -ddNTP terminators.

tors eliminates these background bands even further. More efficient incorporation means less isotope is needed; generally less than one picocurie per sequence is sufficient for overnight exposure. Each set of [α - ^{33}P]dNTPs is function tested with the Thermo Sequenase radiolabelled terminator cycle sequencing kit and pUC18 double-stranded DNA. Both the kit and the isotope packs contain sufficient material to sequence 50 templates.

Reader Enquiry No. 111

Epicentre's new **Ligator Rapid DNA ligation and screening kit** is designed to reduce the time necessary to clone DNA fragments and identify recombinants. With the

Ligator kit, ligation of inserts containing cohesive overhangs or blunt ends can be achieved with high efficiency in about five minutes at room temperature. Ligation of PCR products with A-overhangs into T-vectors can be performed in one hour at 16°C . Following ligation and transformation, this kit can be used for in-well, lysis screening, which allows colonies containing the inserted plasmid to be identified in less than one day, without having to perform DNA isolation or restriction digests.

Reader Enquiry No. 112

Isolation and purification

Geno Technology has launched a new product for the **extraction of proteins and nucleic acids from gels**. The GeneCapsule requires minimal effort and takes 90 s or less. It uses the principle of electroelution and requires no additional equipment: simply pick up the DNA band with GeneCapsule and replace on the top of the gel. Rapid electroelution can recover 10–30 μl of concentrated DNA, as large as 1,000-bp DNA fragments, in as little as 60 s. The recovered DNA is ready for ligation, restriction enzyme digestion, sequencing, amplification, random priming, nick translation and other enzymatic reactions. GeneCapsule is supplied as a kit consisting of 54 units.

Reader Enquiry No. 113

Cambridge Molecular Technologies now offers its Kristal Genomic Mini KG kit designed for the **purification of high molecular weight genomic DNA** from whole blood, cell lines and tissue. The protocol is completely scaleable and the kit can be used to purify DNA from 300 picolitres of blood in Eppendorf tubes to 10-ml of blood in 50-ml tubes. It does not use a binding resin, so shearing of high molecular weight DNA is avoided. With this kit, DNA yields are typically $160\text{ }\mu\text{g ml}^{-1}$ of blood or 8 pg per 10^6 cells. The genomic DNA can be used directly for PCR or restriction enzyme digestion. There is a trial kit of ten samples, as well as kits of 50 and 200 mini-preps, and one for a total of one litre of blood. The company has also introduced three other Kristal mini-prep kits. The Kristal plasmid mini kit includes a 15-min protocol for producing plasmid DNA ready for automated fluorescent sequencing. The yeast kit includes a novel non-enzymatic lysis step for rapid yeast DNA mini-preps. The clean kit is said to offer a quick and simple protocol for PCR and oligonucleotide clean-up.

Reader Enquiry No. 114



Epicentre's ligation and screening kit.

R&D Systems' **Ingenius DNA and RNA molecular-weight markers** have been developed with band sizes that are easy to remember. The DNA markers range from 0.1–12 kb with the 4,000-bp and 500-bp bands present at $3\times$ concentration to allow quick orientation on the ladder. These markers are ready to load and a separate vial of loading buffer that contains tracking dyes is provided for dilution of samples. The RNA markers are provided in two ranges, from 0.1 kb to 1.0 kb and from 0.2 kb to 10 kb. These are supplied in TE buffer and are suitable for use with native or denaturing gels. An RNA template mix is also available for generation of a labelled 0.1–1.0-kb ladder. It comprises seven linearized templates.

Reader Enquiry No. 115

Labwear

Cross-contamination of samples, a problem faced by everyone using PCR, has been addressed by Labcaire with the introduction of its new **laminar air flow PCR cabinet**. The cabinet is designed for PCR and incorporates many features for eliminating the risk of contamination and carry-over during sample and reagent preparation. For greater protection against cross-contamination between samples, the cabinet incorporates built-in ultraviolet lighting to destroy contaminating DNA. The ultraviolet tubes are positioned at the front of the cabinet and controlled by a time switch, bathing the entire working area evenly without causing shadows. The cabinet provides a roomy and comfortable workspace for easy access during PCR operation. The unit also provides a generous 450-mm height for easy sample manipulation and has the overall dimensions of 900 mm $\times$ 630 mm $\times$ 550 mm (H $\times$ W $\times$ D), minimizing the amount of valuable bench space taken up. The combination of HEPA-filtered air and ultraviolet lighting is said to provide improved sample protection by ensuring the destruction of contaminating DNA between different PCR sample preparations. Integrated fluorescent lighting and built-in side polycar-

ADVERTISEMENT

NEWCASTLE UNIVERSITY
MOLECULAR BIOLOGY UNIT



PROTEIN SEQUENCING

Membrane-bound, solid or solution samples

£12 per cycle

5 cycles minimum - no set up charge

For further information contact Dr. Joe Gray
Phone: +44 191 222 8612
Fax: +44 191 222 8129
Email: JOE.GRAY@newcastle.ac.uk

READER ENQUIRY NO. 64

PRODUCT REVIEW

bonate panels, for even illumination over the entire worksurface and glare-free viewing, help to eliminate operator fatigue. Both the ultraviolet and fluorescent tubing are positioned inside the cabinet for easy maintenance. There is a stainless steel worksurface for easier cleaning and greater durability.

Reader Enquiry No. 116

Beckman Instruments has introduced the Microfuge R and the Microfuge Lite personal **microcentrifuges**. The Microfuge R is the first personal refrigerated microcentrifuge to be offered by the company. Its compact size and lightweight design should make it suitable for a wide range of applications, including molecular biology and PCR, as well as in environmental and industrial laboratories. The Microfuge R delivers top speeds of 15,300 r.p.m. with a force of 20,937g. It comes with the F241.5 rotor, which spins 24 1.5-ml or 1.8-ml microfuge tubes. Both offer the advantages of a brushless induction drive and both microfuges are sold with rotors included. The Microfuge Lite weighs only 4.2 kg and has a compact design for routine benchtop separations. It delivers top speeds of 13,000 r.p.m. and a force of 12,054g. This microcentrifuge is shipped with a versatile fixed-angle rotor that runs 18 1.5-ml or 1.8-ml microfuge tubes or 18 of the 400- μ l tubes.

Reader Enquiry No. 117

Robbins Scientific now offers the TruTemp DNA **microheating system**. Composed of a heated chamber and a lid, this new instrument is specially designed to provide fast heat up and consistent controlled temperatures. This dual heating system denatures DNA samples at 95 °C from ambient in less than 12 minutes. For hot-start PCR protocols, reaction mixtures can be quickly and precisely heated to the starting temperature, maintained for the addition of the last reagent, and then moved to the thermocycler. With consistent temperature control, the company says the system can provide hours of incubation of probe solutions with target cells at the selected temperature for *in situ* hybridizations. Small and lightweight, TruTemp measures 14 cm $\times$ 30 cm $\times$ 20 cm and weighs less than 2.3 kg. For added versatility, heating blocks are available for standard 0.6- and 1.5-ml microcentrifuge tubes, 0.2-ml microtubes and standard glass slides. The combination of quick heating, precise temperature control and an assortment of heating blocks should make this unit a useful addition to the molecular biology laboratory.

Reader Enquiry No. 118

The new Savant GTF100 **electroporator** features three selectable settings and a short recharging time for rapid processing of samples. Designed to take some of

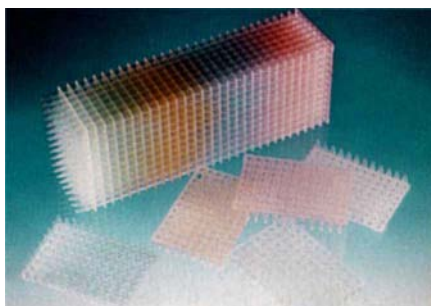


Savant's GTF100 electroporation unit.

the guesswork out of electroporation, the company says this unit provides consistently high transformation efficiencies for both bacterial and mammalian cells and has a recharging time of less than ten seconds. Optimum settings are provided for bacteria, such as *Escherichia coli*, in either 1-mm or 2-mm cuvettes. A third setting accommodates the conditions for mammalian cells using 4-mm cuvettes. The GTF100 is compact and lightweight, allowing users to move it and store it without difficulty. It will accommodate all standard electroporation cuvettes currently on the market. High quality sterilized cuvettes are also available in three convenient gap widths (1- and 2-mm for bacteria and 4 mm for mammalian cells).

Reader Enquiry No. 119

Advanced Biotechnologies has introduced the Thermo-Fast one-piece, injection-moulded, V-bottomed **96-tube plate for thermal cycling** applications. This improved version offers the advantages of a thin-walled tube, in addition to handling convenience — important characteristics for high-throughput laboratories. The plates can be sealed using the company's domed Micro-Caps, adhesive



Thermo-Fast V-bottomed tube plates.

films, Micro-Mats or with Thermo-Seal heating/sealing foils. The raised-rim design is said to be particularly suited to one-piece sealing devices as it forms a better contact compared with polycarbonate alternatives, eliminating the problems of leakage and cross-contamination. The tight sealing of the plates enables oil-free amplification, so reaction vol-

umes as small as ten picolitres can be used, saving on expensive reagent costs. Thermo-Fast can easily be cut into sections to fit various block formats, such as 3 $\times$ 8, 5 $\times$ 5 or 6 $\times$ 8. The plates are also available pre-cut.

Reader Enquiry No. 120

These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.

ADVERTISEMENTS

LIPOSOMES

REPRODUCIBLE — HOMOGENEOUS —
EXTREMELY RAPID



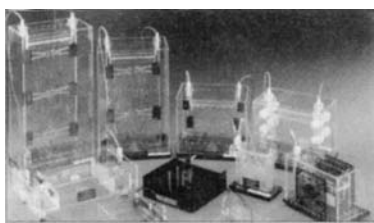
Our new LIPOSOMAT guarantees rapid (5ml within 60 minutes) preparation of uniformly sized unilamellar liposomes. Liposome size is selectable between 25 and ca. 600 nm diameter. Sample volume is normally 5-10ml or up to 200ml if used in combination with an additional instrument. Lipid concentration can be up to 300 mg/ml.

DIABORM

P.O. Box 650126, D-81215 Munich
For USA call 1-800 Liposome

READER ENQUIRY NO. 63

**DNA & PROTEIN
ANALYSIS GEL UNITS**



- Vertical
- Horizontal
- Mini submarine
- Fan cooled
- Water cooled
- Double sided
- 2 Dimensional
- Wet blotter
- Semi Dry blotter
- Custom

- SIMPLE —
- SAFE —
- EFFECTIVE —

CAMBRIDGE 84 High Street, Cherry Hinton
Cambridge, England CB1 4HZ
Telephone: (01223) 249431
Fax: (01223) 411803
ELECTROPHORESIS LTD

READER ENQUIRY NO. 62